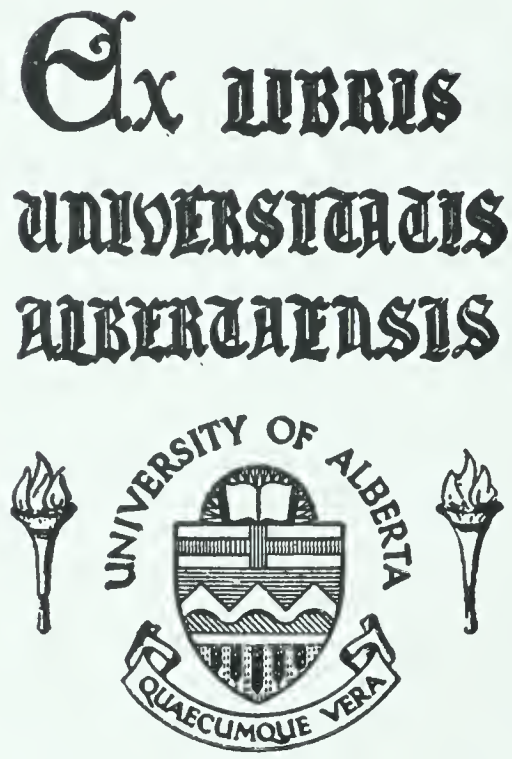

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POOR GROWTH OF ALFALFA IN THE STONY PLAIN AREA ON CERTAIN SOILS THAT HAD PREVIOUSLY GROWN THIS CROP

by

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A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Poor growth of alfalfa in the Stony Plain area on certain soils that had previously grown this crop" submitted by Shahamat Ullah Khan, B.Sc., M.Sc., in partial fulfillment of the requirements for the degree of Master of Science.

ABSTRACT

A serious problem encountered by some farmers in the Stony Plain area is that alfalfa grows poorly on land that had previously grown this crop. The plants in areas of poor growth are usually spindly, yellowish, and poorly nodulated and fail to make sufficient growth for even one cut. In less severely affected areas growth is patchy. During 1960 to 1962 greenhouse (24) and field (65) experiments were conducted on several of these problem soils. The results suggested that a biological factor, in addition to mineral nutrients, in at least some of the soils was partially responsible for the poor yield. Subsequently the study was expanded to obtain more information, particularly regarding this biological aspect. Soils from three farms, namely Nonay (Leith sandy loam), Webber (Peace Hills fin sandy loam), Boje good (Leith sandy loam) and Boje poor (Codner sandy loam) were collected. Treatments consisting of fertilizer, partial and prolonged steaming, vapam, formaldehyde, and nemagon were used.

Steaming the soils resulted in marked and prolonged yield increases for alfalfa and barley. This effect was greatest for the soils with the highest organic matter contents. Steaming also produced more branched and prolific root systems of alfalfa than other treatments. Vapam markedly increased alfalfa yields for the Nonay soil. Other chemical treatments resulted in variable responses. Applications of macro- and micronutrients increased yields but not nearly to the same extent as steam treatments of Webber, Boje good and Boje poor soils, and vapam treatment of Nonay soil. The data support the conclusion from earlier studies that the poor growth of alfalfa is associated with some factor or factors in

addition to those associated with mineral nutrition.

The number and weight of nodules, and haemoglobin concentration appeared to be greatly increased by vapam treatment. For the Nonay soil these properties were significantly correlated with yield of alfalfa. Rhizobium strains from plants grown in treated soil were more effective than the check as indicated by increased growth when the various nodule cultures were applied to alfalfa grown in sterilized sand.

Partial and prolonged steaming did not completely sterilize the soils but changed the composition of the microflora markedly. Vapam eliminated the fungi in all soils. Partial and prolonged steaming, vapam, and formaldehyde treatments prevented nitrification in the soils. After six weeks in the greenhouse, however, active nitrification was again taking place.

In summary, the data suggest that some biological factor in addition to mineral nutrition is involved but further studies will be required to isolate and identify the agent. Since all soils were not affected alike by the treatments, particularly steam and vapam, it appears that the reactions associated with this factor are complex.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The nitrogen status of soils usually undergoes a gradual deterioration when land is first used for agriculture. Cultivation disturbs nitrogen equilibrium and the content usually decreases when land is broken and in later years it may stabilize at a lower level. Modern agricultural development such as growing of row crops instead of grasses and legumes, introduction of high-yielding varieties, and intense cultivation practices have also contributed to this lowering.

The problem of maintaining nitrogen supplies for crops may be approached in a number of ways. Nitrogen may be added through the fixation of atmospheric nitrogen by legume bacteria and by non-symbiotic nitrogen fixing organisms. It may be added in rain water or snow or supplied in the form of artificial fertilizers, farm manure, crop residues, compost, etc.

Nitrogen economy through symbiotic fixation by leguminous plants has been emphasized in several comprehensive surveys. Walker (92) has stated that in New Zealand legumes fix sufficient atmospheric nitrogen to meet the needs of pasture crops. Lipman and Conybeare (43) estimated that for the year 1930 the 387 million acres sown to legumes throughout the United States accounted for about 1.7 million short tons of nitrogen fixed, or about 87.6 pounds of nitrogen per acre. Only six pounds of nitrogen per acre were attributed to non-symbiotic nitrogen fixation. McKee (52) estimated that in 1948 within the United States the acreage of all legumes cut for hay was 40 million acres; cut for seed, 15 million acres; used for clover, 5

million acres and in pasture, 40 million acres. On the ultra-conservative basis of a ton per acre dry weight of tops and roots averaging 2 per cent nitrogen, the total nitrogen fixed would be more than 2 million tons. At that time, the total nitrogen used annually as commercial fertilizer in the United States was less than 500,000 tons.

The use of a legume forage in a crop rotation is necessary if a permanent agriculture is to be achieved in many areas of Alberta. Alfalfa has been considered one of the best legume crops and provides an excellent source of protein for livestock. In the Stony Plain area a serious problem encountered by some farmers is that alfalfa grows poorly on land that had previously grown this crop. Several fields of established alfalfa have been examined during the last few years and it has been verified that the problem is real and important.

A greenhouse study was begun in the fall of 1960 by Goettel (24). Soil samples were collected from four farms where alfalfa growth had been poor and from one farm where growth had been good in recent years. The most interesting feature of this greenhouse study was the very marked increase in yield from partial steam treatment. This response was much greater than those to the application of macro- and micronutrients. It suggested that a biological factor in at least some of the soils may have been partially responsible for the poor yield.

In the spring of 1961, experiments were placed on four established alfalfa fields to determine the response to applications of fertilizer. The effect of additions of water on the growth of alfalfa was also

studied. It was noted that the yields at locations 1-51-27-4 (Boje), 34-50-27-4 (Nonay) and 13-53-28-4 (Webber) were low. The soils at these three locations are coarse textured. The yield at location 2-53-27-4 (Schwindt), situated on a fine-textured soil, was much higher than for the other three. The latter soil has consistently produced good stands of alfalfa. The poor yield on the other soils suggested that some factor other than moisture and nutrients was limiting yields. The greenhouse experiment also supported this conclusion.

In the spring of 1962, there were very striking growth differences of alfalfa in various parts of a field at location 1-51-27-4 (Boje). Alfalfa had grown well in this field during the first time in the rotation some eight or nine years previously suggesting that the problem was the same as the one discussed in the foregoing paragraphs. In the poor areas, growth was very stunted and yellowish while in other areas it was good and plants were well nodulated. There appeared to be some relationship between topography and vigor in that growth was generally poorest in the low areas and best on the high areas in the field.

In view of the seriousness of the problem and using the information collected by Goettel in the preliminary study, the investigation was expanded to study, in particular, the effects of certain partial sterilization treatments. The problem was acute at locations 1-51-27-4 (Boje), 34-50-27-4 (Nonay) and 13-53-28-4 (Webber). Using soils from these areas, this investigation was undertaken with the following objectives:

(a) To study the effect of different sterilization and fertilizer treatments on yield, plant nitrogen and phosphorus content, root develop-

ment, nodulation, concentration of haemoglobin in nodules, and production of effective and ineffective rhizobium strains.

(b) To investigate the relationship between nodule weights, nodule number, haemoglobin concentration and the yield of alfalfa.

(c) To investigate the effect of different sterilization treatments on microbiological activity (respiration rate, etc.), nitrate-supplying power of the soil, and the extent to which the soil is sterilized.

(d) To investigate whether a toxic substance or a microbiological balance was responsible for the poor growth of alfalfa in some parts of the field at location 1-51-27-4 (Boje).

(e) To study whether the different sterilization and fertilizer treatments affect alfalfa and barley the same way when grown in a soil from location 1-51-27-4 (Boje).

REVIEW OF LITERATURE

Nitrogen Fixation by Legumes

Empirical knowledge of the soil-improving value of leguminous plants in agriculture extends back to ancient times, but only since 1886 has the mysterious nature of this beneficial effect been replaced by precise knowledge. In that year Hellriegel and Wilfarth in Germany first showed conclusively by quantitative experiments that legumes are able to assimilate atmospheric nitrogen. There ensued a great wave of interest in the subject. Beijerinck in 1888 was the first to isolate the bacteria from nodules and grow them in pure culture. Throughout Germany, France, Italy, Holland, and England, intense interest was taken in the subject during the following three decades and many and varied aspects of symbiosis were closely studied. Following the 1914-18 war, the focus of interest moved to North America where several influential centres of research on Rhizobium came into existence. More recently some workers in southern Australia have become interested and several centres of Rhizobium research are now active in Australia.

The amount of nitrogen fixed by a leguminous crop depends on several characteristics of the nodules including their number, size, longevity, haemoglobin content, and the efficiency of fixation of the various bacterial strains in the individual nodules. It also depends on the strain of legume, conditions of growth and management of the crop, and in particular on availability of water and nutrient status of the soil. It is reasonable to expect that the rate of nitrogen fixation would be dependent on the total volume of active nodular tissue the crop is carrying, but very few field observations have been made on this aspect.

Jones (34) has studied the effect of soil temperature on the development of nodules of alfalfa and reported that the temperature range which often occurs in cultivated fields affects the initiation and development of nodules on the roots of alfalfa to such a degree that assimilation of nitrogen is greatly modified during the summer. Masefield (48, 49, 50) conducted studies on the weight of nodules carried by some crops both in temperate and tropical regions although he observed the weight of nodules per plant rather than per acre. He concluded that generally certain leguminous plants grown in Britain had a higher weight of nodules per plant than when grown in Nigeria possibly because of the lower soil temperatures and higher soil moisture conditions in Britain. Furthermore, plant weights were found to be significantly correlated with nodule weights in the case of field beans and peas (48).

Different levels of many inorganic nutrients alter nodule development in one of several ways. MacConnell and Bond (28) observed the stimulating effect of small additions of nitrogen on nodulation. It can be seen from their data that there was a significant increase in the number of nodules per plant at all levels of NH_4-N supplied, ranging from 10 to 100 mg. per litre of culture solution. Richardson et al. (66) have also shown that supplying nitrogen in the ammonium form at the rate of 0.5 to 12 ppm. increased nodulation markedly in Ontario Variegated alfalfa, but decreased nodulation when applied as nitrate-nitrogen particularly at high rates (60 ppm.).

An abundance of nodules on the root system of the host is not necessarily a criterion of successful nitrogen fixation and it would

appear that in order to truly evaluate the effectiveness of root nodules in nitrogen fixation, it is necessary to proceed further than the commonly accepted methods. A direct relationship has been found by Virtanen (85, 86) between the concentration of haemoglobin and the amount of molecular nitrogen fixed by symbiosis. This correlation has been corroborated by Smith (75) who reported that the concentration of haemoglobin in the bacteria-containing cells of nodules of different legume species varied between 1 and 5 x 10^{-4}M. Virtanen and Laine (84) have shown that it is possible to estimate approximately the nitrogen fixing efficiency of a root nodule in cultivated soil simply by slicing the nodule and examining the color of the cut surface. If the color is red the activity is high, if brown the activity is lower and if green it is nil and the nitrogen fixation is irrevocably at an end.

Jordan and Garrard (35) reported that maximum haemoglobin concentrations occurred just prior to blossoming in field legumes, but much earlier than this stage for greenhouse legumes under the conditions of their experiment. The nodules of the ineffective strains of legume bacteria differed from those produced by effective strains in color, size, distribution, rate of destruction of the bacteriod area, and presence or absence of rod-like bacterial forms. They have also presented experimental evidence to show that a strain of alfalfa Rhizobium may function parasitically under greenhouse conditions but this has not been observed under field conditions. The use of field trials to substantiate greenhouse results is, however, contraindicated by the large number of soil factors tending to influence fixation; nevertheless, it seems plausible that any strain of nodule bacteria which acts as a parasite under controlled conditions is potentially capable of similar action in the field.

Effect of Bacteriophages on Root Nodule Bacteria

While the first hint of phagic activity upon rhizobia may have been suggested in the frequently cited paper of Loew and Aso (44) definite interest dates rather sharply from the combined report of Gerretsen and Sack of Groningen and of Solingen and Giryns of Wageningen. In 1923 these investigators, each pair working independently, obtained lytic agents from nodules, roots, and stems of clover, lupine, and serradella plants, and from garden and field soils. Their description of lytic action by these agents leaves little doubt as to their bacteriophagic nature. The importance of bacteriophage in agriculture was recognized in the early forties. Razumouskaya (64) was the first to show that despite the subsequent development of resistant forms, the addition of phage to soil accounted for a decrease in the population of alfalfa rhizobia, as evidenced by depressed nodulation and subnormal yields of alfalfa. The same year marked the beginning of a long series of papers by Demolon and Dunez (16, 17) emphasizing the phage as the major cause of "alfalfa fatigue".

The thesis of these French workers warrants exposition here, since it embodies comprehensive laboratory and field tests over a 15-year period. According to them, "alfalfa fatigue" as manifested by lack of plant vigor, pale green foliage, small ineffective nodules, and reduced crop yield of low quality, is the inevitable result of repeated production of this crop in phage-infected soils. In 1934, Demolon and Dunez (16) reported that they had obtained proof that in "sick" soils, Bacillus radicicola[1] had completely disappeared owing

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[1]Syn. Rhizobium.

to the effect of bacteriophage. In 1935 on the basis of pot and field experiments in which inoculated seed was sown into bacteriophage-infested soils, they concluded that the phage retarded nitrogen fixation by the root nodules. They isolated a bacteriophage from the soil surrounding the roots of old alfalfa plants and concluded that it interfered with the symbiosis between the rhizobia and the host plant.

The cycle of the phage, as gleaned from their studies, consists of several distinct stages: (a) a period of low incidence attributed to a lag phase as affected by the quantity of host cells present, (b) passage of the phage from the soil into the nodules, where it increases in quantity and potency and thence into the root tissues, and (c) its ultimate release into the soil during periods of nodule decay, sloughing off of root tissues, and decomposition of the entire root mass. Disappearance of the phage from the soil in the absence of the host plant is explained by its sensitiveness to desiccation, insolation, irrigation, anaerobiosis, low temperatures, and its adsorption and inactivation by a variety of soil substances.

Four remedial measures for alfalfa-fatigued soils are suggested by these workers, namely: (a) temporary abandonment of alfalfa culture in the same field, (b) the substitution of a different crop, preferably a nonleguminous one, (c) the selection of a phage-resistant alfalfa variety or species, and (d) the application of inocula consisting of polyvalent resistant rhizobia strains. Considerable supporting evidence is supplied by Vandecaveye and coworkers in the study of alfalfa culture in the state of Washington. Vandecaveye and Katznelson (82) reported that soil extracts obtained from a three-year-old stand

of alfalfa contained a lytic principle that was strongly active against two of five different strains of Rhizobium meliloti and fairly active against two others and inactive against a fifth. The roots of the alfalfa carried few nodules but all those tested produced lysis. They found that bacteriophage was present in several soils carrying old alfalfa stands but not in soils growing alfalfa less than three years of age. All nodules, however, showed a lytic action and this occurred regardless of the age of the plants carrying the nodules. These workers concluded with the possibility that a bacteriophage was responsible for reduced yields of alfalfa but that definite conclusions were reserved pending further investigation.

Demolon and Dunez (17) reported that it was possible to obtain strains of nodule bacteria resistant to lysis by bacteriophage and also very effective in nitrogen fixation. Field experiments on alfalfa "sick" soils showed considerable advantage in the use of such strains. Katznelson and Wilson (37) found R. meliloti phage in the soil from all the alfalfa fields that they tested in the state of New York. They considered it to be a normal condition which is contrary to reports of other investigations. In addition, they reported no serious interference of the phage with symbiotic nitrogen fixation. There was no correlation between age of stand, soil type, pH, and phage incidence. They indicated, however, that certain fertilizer treatments may be conducive to phage growth but did not have sufficient information for conclusive results. These workers felt that the phage was present in all soils but only under certain conditions did it become sufficiently strong or potent to destroy rhizobia and interfere with

symbiosis. Servici de Rondini (71) conducted detailed studies of alfalfa plants and soil extracts from areas of failing alfalfa crops and found no evidence that the anti-rhizobial factor present was of the ordinary bacteriophage type. He found this factor present in soil extracts but not in alfalfa root extracts.

Kleczkowska (39) reported that bacteriophage for clover nodule bacteria can be found on roots and nodules of all naturally grown clover plants and also in the soil surrounding the roots but not in soil without clover plants. Alternative hosts for the phage of clover nodule bacteria are pea nodule bacteria in the sense that only a proportion of strains of clover bacteria and pea bacteria are susceptible to lysis by a given race of phage and only a proportion of races of phage can lyse a given bacterial strain. Further she stated that there does not seem to be any association between the susceptibility of bacterial strains to lysis by phage and any other features such as antigenic structure or effectiveness in nitrogen fixation. There may be an association with avirulence, i.e. inability to infect the host plant. As long as the phage is present, phage-resistant bacterial mutants are also usually present which may also be mutants in other respects such as effectiveness in nitrogen fixation. Kleczkowska found that in the presence of weakened phage, bacterial mutants differ from the parent form in effectiveness but resemble it in susceptibility to the phage.

Effect of Different Fertility Levels on the Growth of Alfalfa

The fertility status of soils has an important bearing on the growth of alfalfa. Although alfalfa will supply its own nitrogen needs, its

requirements for phosphorus, potassium, calcium, magnesium, and other essential elements are substantial. Unless strict attention is given to the mineral nutrition of this crop, the maintenance of a good stand for more than a few years is often impossible. Brown (9) found that nitrogen applications tended to increase weed growth and decrease the stand of alfalfa. In a preliminary study conducted by Purvis (63), 25 and 50 pounds of applied nitrogen per acre reduced the yields and a heavy application was beneficial only if the alfalfa was cut four or more times annually. Gerwig and Ahlgren (23) reported that in the year following seeding the application of 25 pounds per acre of nitrogen was beneficial and increased the yield and the percentage nitrogen in the plant. Higher rates were detrimental to nitrogen fixation. Fifty pounds per acre resulted in lower yields and lower nitrogen contents and an application of 100 pounds or more per acre gave yields equal to the 25 pound application. In succeeding years, nitrogen fertilization tended to decrease the yield and the stand and to increase weeds.

Research studies in the northeast section of the United States show few benefits from the addition of phosphorus to alfalfa (9). However, in extensive areas of the West and in limited areas of the humid regions where marked phosphorus deficiencies occur in the soil, applications may be beneficial. No significant increases in yield or persistence of alfalfa during a three-year period were realized by phosphorus fertilization (23) but each increment of phosphorus applied increased the per cent phosphorus in the plant. Alfalfa responded markedly

to applications of phosphorus for some soils of Alberta (65). Gross et al. (26) found that high rates of potash were necessary in order to maintain alfalfa stands and to consistently produce high yields. Results of a study by Wang et al. (87) on the effect of lime and fertility levels on the winter survival of alfalfa in Wisconsin left no doubt that high levels of lime and available phosphorus and potassium, particularly the latter, promoted winter survival of alfalfa. According to Twamley (78), the addition of potassium almost invariably resulted in more persistent stands and in higher yields. Its beneficial effect was most noticeable when the alfalfa was under stress caused by improper management and/or lack of resistance due to an unfavorable environment. For some Alberta soils, it was shown that alfalfa generally responded to applications of nitrogen and phosphorus in combination, whereas the responses to potassium were small (65). Gerwig and Ahlgren (23) recorded an increase in yield with each increment of potash applied up to the 200-pound rate. There was also an increase in per cent potassium in the plant with each increment of potash applied. Potassium deficiency decreased the stand by as much as 80 per cent on those plots not receiving potash.

The concept of "critical percentage" of each essential element has been applied to many crops, including alfalfa. In severely stunted alfalfa phosphorus levels as low as 0.10 to 0.12 per cent have been reported in the literature. However, it seldom falls below 0.15 to 0.17 per cent. The "critical percentage" set by Bear and Wallace (7) was 0.27 per cent. The "critical percentage" of potassium in alfalfa was found to vary from season to season, and probably lies between 1.42 and 1.84 per cent (7, 23). Under normal management practices,

the potassium content of the plant needed for the survival of alfalfa was found to be 1.00 per cent.

Because of "cation equivalency" in plants, a reduction of calcium content will result in a complementary increase in potassium uptake. As the potash application increased higher potassium content and lower magnesium and calcium contents were found in the plant (23). It would, therefore, seem advisable in growing alfalfa to keep calcium and magnesium at the proper level and apply increments of potassium annually as needed.

Soil Moisture and Alfalfa Production

Kiesselbach et al. (38) reported that alfalfa produced well during the first three or four years on land growing this crop for the first time in Nebraska. During this initial period yields were practically independent of the amount of rainfall. Thereafter, they declined and became largely dependent upon precipitation. On land that had previously grown alfalfa, even as long ago as fifteen years, the yields appeared to be dependent upon rainfall from the beginning and were never as high as during the early years on land where this crop had not previously grown. This reduction in yield could not be due to a depletion of mineral nutrients as treatments using manure, commerical fertilizer, gypsum, and lime had little benefit. They concluded that the yield reduction was due to moisture deficiency.

Some investigators (25) have suggested that alfalfa plants would have to depend entirely on seasonal rainfall for their moisture needs, unless a period of summer fallow preceded the planting of this crop on land which had previously grown this deep-rooted legume. It was

concluded that this was true even when a considerable number of cereal crops was grown on the land between the alfalfa plantings. That moisture is frequently a limiting factor in the growth of alfalfa has been confirmed by various other workers.

Effect of Microbiological Activity on Soil Fertility

Studies in soil microbiology have served to emphasize the important role played by microorganisms in the development and maintenance of soil fertility. Through the use of improved laboratory techniques and the introduction of standard methods of procedure, more concordant results have been obtained and these have led to the conclusion that the activities of soil microorganisms may serve as an index of fertility. The accumulation of nitrate in soil, when incubated under optimum conditions of temperature and moisture for a given length of time, has been used as a criterion for determining the nitrogen-supplying power of soil by several investigators. White et al. in Pennsylvania (93) have reported a highly significant correlation between crop yields and nitrifying capacity of soils. Hanway and Dumenil (29) in Iowa found a significant correlation between nitrification rate and the response of corn to applications of 40 and 60 pounds of nitrogen per acre. Allen and Bonazzi (3) found that nitrifying capacity of a soil may or may not correlate with its crop-producing power. Synghal (76) reported that, of all the methods tested for assessing the nitrogen requirements of Alberta soils, a nitrate accumulation method seemed to be the best. This test gave a highly significant correlation with check yields in the greenhouse, while total soil nitrogen and nitrogen in the unfertilized plant material did not correlate with

yields. Nitrate initially present in the soil and the per cent of total nitrogen mineralized to nitrate were also found to be comparatively poor tests. Eagle and Matthews (19) have evaluated the various factors affecting the results of the incubation method for measuring the nitrate-supplying power of soils and yield of crops grown in the field. The nitrifying capacity of soils with a few exceptions seems to be quite a useful test for determining the nitrogen fertilizer requirements of soils.

The numbers of microorganisms in a soil give no direct measure of the activity of the microbiological population. The activity of the population is a concept that cannot be given a quantitative definition, but for many purposes it can be measured by the amounts of CO_2 evolved by the soil. In general, this evolution increases as the number of bacteria increase. Some workers have found a close relationship between bacterial numbers as determined by the direct counting and CO_2 evolution at various seasons of the year for different soils, and concluded that activity of the whole population rose and fell with the numbers of bacteria so determined. However, CO_2 evolution was poorly correlated with the bacterial numbers when the pour-plate method was used. The only condition when fungi seemed to contribute appreciably to CO_2 evolution was when vigorous growth of mycelium occurred on buried Cholodny slides, and this occurred for only one or two weeks after fermentable organic matter had been added to the soil. It has been reported by Russell and Russell (70) that pour-plate counts are not well correlated with total soil respiration, whereas direct counts may be well correlated. Oliver (59) has reported that decarboxylases remain active after the cells

are killed by exposure to 80°C. for 5 minutes or momentary exposure to 100°C, which supports the hypothesis of heat-stable enzymes in the soil. His finding that heat stability of these enzymes is increased in complex media indicates that enzymes may be protected from heat by soil organic matter. It has been shown that, in the respiration of soil, some activity must be due to residual enzymes associated with organisms incapable of multiplication on the medium provided in the plate count technique and this provides a further explanation for the low correlation between plate count and respiration and the high correlation between total count and respiration (10, 53, 61).

Effect of Soil Sterilization on Chemical and Biological Properties of the Soil

The occurrence of various pests in the soil, namely pathogenic fungi and bacteria, nematodes, insects, and weeds have reduced in certain cases the productive capacity of vast areas of otherwise good land. Soils can be rid of these pests by partial sterilization with steam, chemical fumigants, or disinfectants. Partial sterilization often has a beneficial effect on plant growth, and has become a wide-spread practice in the greenhouse. Partial sterilization is known to affect the physical properties of the soil, to increase the solubility of many nutrients, to cause profound changes in the microflora and fauna and to kill weed seeds.

Steaming soils in now a normal practice in many greenhouse operations and it has two advantages over most of the other methods in that it is non-selective thereby killing all pests and it does not leave a toxic residue. Not all the consequences of steam treat-

ment are yet understood. Malowany and Newton (46), in their study of the changes caused by steaming four Alberta soils, reported little change in the physical properties. They did, however, obtain a significant decrease in the capillary rise of moisture for the four soils, and the decrease was greatest in the high organic soils and least in the low organic soils. They also noted that steam-treated soils often had a lower water-holding capacity than untreated soils. Van Bavel (81) and Lawrence (41) have reported increased soil aggregation after steam treatment. The effect of steam sterilization on pH of soils has not been extensively investigated, but Malowany and Newton (46) and Davis and Owen (14) found that it had little or no effect. Steam under pressure can cause an increase in the concentration of soluble salts in the soil. Newhall (56) has reported twofold to tenfold increases in soluble salts in steamed greenhouse soils; he also noted that soils rich in organic matter had a higher total salt content after steaming than soils low in organic matter. Furthermore, a higher concentration of Ca, P, K, Mg, Zn, Mn, and Cu and a lower concentration of Fe occurred in plants grown on steam-sterilized soil. Malowany and Newton (46) found that water-soluble phosphate was greatly increased and water-soluble sulphate somewhat increased by steam sterilization, the increases being greatest in soils rich in organic matter. Walker and Thompson (91) noted that steaming has a big effect on the amount of water-soluble oxidizable organic matter present in soil. Aldrich and Martin (1) found that steam sterilization produced substantial changes in the amount of Ca, Mg, K, and Mn in the extract of treated soils. Fujemoto and

Sherman (21) and Walker and Thompson (91) also found that steaming increased the amount of water soluble Mn, particularly in acid soils. If these soils were limed before treatment, there was a big reduction in the amount of Mn obtained. Lapensee (40) found that steaming increased the solubility of Ca, Na, Mn, Mg, S, and P in soils whereas potassium remained unchanged. He noted that soluble or exchangeable soil manganese was highest in steam-treated soils, and least in the untreated check. Liming resulted in a decrease in available manganese proportional to the amount of lime added and inversely proportional to the soil moisture content. Steam treatment causes a rapid rise in ammonium production, which may or may not nitrify after several weeks, probably depending on how soon and how effectively nitrifiers are introduced into the soil (13, 14). Steam destroys or temporarily inhibits the action of nitrifying bacteria in soil. The transformation of ammonium to nitrate, normally accomplished by these bacteria, is thus delayed. Ammonifying organisms including spore-forming bacteria, however, escape destruction by the partial sterilization, and consequently ammonification may proceed uninterrupted. After the onset of nitrification, the ammonium concentration falls to a normal level. Waksman and Starkey (88) and Davies and Owen (13) found that some ammonium was formed during the process of sterilization, but generally there was no sudden increase in ammonium concentration, but rather a gradual increase with time (13, 14, 91). The amount of ammonium ultimately formed is determined largely by the organic matter content of the soil, increasing with increased organic matter (46, 72), or the amount of nitrogenous fertilizer added (77).

A second steaming usually gives less ammonium than the initial treatment, particularly if performed soon after the first (13). Davis and Owen (13, 14, 15) studied ammonium production and subsequent nitrate formation in several greenhouse soils following partial sterilization by steam. They found for soil steamed in situ and left undisturbed apart from watering, that ammonium production continued for a considerable time and nitrate formation was negligible despite the fact that the soil was open to aerial or other fortuitous infection. About three months after steaming, the ammonium slowly began to decrease, but a further three to four months elapsed before it fell to normal levels. Regular turning of the soil, or the planting of a crop however, greatly hastened the onset of nitrification when compared with undisturbed steamed soil. Mixing of steamed with untreated soil also reduced the period of ammonification. Thus, where the soil was used and handled in the ordinary processes of potting and planting little build-up of ammonium occurred (90).

Cases are known, particularly after soil steaming, where growth is depressed and, in extreme cases, serious damage caused. It has been thought (33,69) that ammonium, the production of which is usually greatly increased by partial sterilization, is the most likely toxic agent but other products such as excess soluble salts or excess soluble manganese may be partly responsible (40). Davis and Owen (13) concluded that the high ammonium concentrations induced by steaming are not toxic to plants and quote supporting data of Read

and Shead who added 412 ppm. of tribasic ammonium phosphate to steamed soil and found that the resulting mixture had no deleterious effect on tomato and other seedlings. Robinson (67) also came to the conclusion that ammonium was not responsible for the inhibition of growth occurring in steamed soil. His evidence indicated that the toxic factor was not excess NO_2^-, Cl, Mn, Al, Fe, or total soluble salts. The deleterious effect was overcome by heavy applications of phosphate.

Steam sterilization of soils eliminates soil borne plant pathogens and creates a new medium of special composition for the surviving micro-population or the organisms gaining access from external sources. When soil is treated with steam, bacteria are reduced in number for a period, then multiply rapidly, usually to numbers exceeding those in the untreated soil (51, 68, 77, 88). After reaching a maximum, bacterial numbers fall towards those of the untreated soil (51, 88). The fall is sometimes very slow, taking over a year. The effect on the fungal population, however, is much more marked and the range of species and numbers present, may be depressed for 12-18 months, even if fungi are introduced into the soil (74). Katznelson and Richardson (36) found that certain species of Penicillium increased in some soils after partial sterilization with steam. Ludwig and Henry (45) observed that steam-treated soil reinoculated with untreated soil contained large numbers of Trichoderma. Baker (6) quoted Warcup's data regarding the effect of aerated steam treatment of soil at various temperatures on the fungus flora. The number of fungus species determined were as follows:- untreated check, 30; 120°F., 31; 130°,

11; 140°, 7; 150°, 7; 160°, 3; 170°, 3; 180° and above, 0. Actinomycetes were plentiful up to 100°F.; bacteria were abundant up to 180°, and higher temperatures were not tested. Evans (20) studying fungal recolonization of steam-sterilized soil in special recolonization tubes, found that if the soil was loosely packed Trichoderma was almost always the leading fungal colonizer; but if the soil was closely packed Phycomycetes, Phythium, Zygorrhynchus, and Mucor were among the leading colonizers. Singh and Crump (73) concluded from their results on the occurrence of amoebae in soil after partial sterilization with steam that no generalization can be made on the growth and multiplication of amoebae in field soil. They found with steam treatment that after an initial drop the number of bacteria and amoebae rose higher than in the untreated soil.

The effect of partial sterilization with chemicals is somewhat similar to that by steam and a number of chemicals have been used in the past 30 years as soil fumigants. The soil pests are presumably killed by action of the fumigant on the protoplasm of the cells. Chloropicrin destroys all types of protoplasm, plant as well as animal. D-D mixture, ethylene dibromide, nemagon, ve-13 nemacide and telone act only on animal protoplasm, specifically that of nematodes. Methyl bromide shows promise for control of nematodes, weed seeds and many fungi, while vapam and mylone are said to control nematodes, certain fungi, and weeds. Terraclor acts only as a fungicide.

Formaldehyde, usually applied as formalin (40% formaldehyde in aqueous solution), is useful against certain fungal pathogens and is occasionally used as a general sterilizing agent (27).

Lawrence (41) reported a loss of soil structure when formaldehyde was used as a fumigant since large amounts of water are used as a vehicle for the chemical. Dalton and Hurwitz (12) have noted an increase in solubility of soil Mn, sometimes to toxic levels, after formalin treatment. Many workers have reported that this treatment destroys the nitrifying bacteria in the soil, while Tam and Clark (77) have found nitrification was inhibited little. Katznelson and Richardson (36) showed that formalin had a slight reducing effect on actinomycete population.

More is known, both quantitatively and qualitatively, of the effect of partial sterilization on fungi than on any other group of organisms in soil. It has been found that a large proportion of the fungi in soil is killed by partial sterilization, the number destroyed depending on the treatment and dosage (47, 51, 77). Mollison (55) found that formalin treatment markedly reduced the fungal flora of an old forest-nursery soil in the field. He also found that fungal recolonization of the treated soil was slow, and even 18 months after treatment the number of species and colonies was lower than in untreated soil. This slow recolonization in the field is in direct contrast to most other work where the total number of fungi increased greatly at varying periods after partial sterilization. Katznelson and Richardson (36) found that certain species of Plectonaemella increased in some soils after partial sterilization with formalin. The resistance of Trichoderma to this chemical has been studied by many workers (20, 55) who found its tolerance to formalin both in soil and agar to be outstanding. Garrett (22) has pointed out that one of the most promising approaches to the problem of biological

control appears to be through the alteration of soil conditions by partial sterilization. The complexities of this approach, however, are shown by Garrett(22) who reported that quite contrary to expectation, percentage incidence of seedling damping-off from re-introduced inoculum of Pythium ultimum into formalin-treated soil was highest in those treated soils characterized by the most pronounced dominance of Trichoderma viride. A reasonable explanation for this was obtained by Smith (74) when he found that the isolates from this particular soil after formalin treatment produced neither gliotoxin or viridin.

Vapam, a water-soluble soil fumigant, is a new temporary soil sterilant (60). It is highly effective in control of weed seeds, nematodes, fungi, and also shows promise for control of several species of soil-infesting arthropods such as wire worms, grape phylloxera, garden centipede, and the bulb mite. Nemagon, another soil fumigant is used extensively for the control of nematodes (62). Carbon disulphide, orginally used extensively against phylloxera and other insect pests, has also been used against Armillaria root-rot of citrus. Russell and Hutchinson (68) found that soil treated with carbon disulphide was able to supply plants with more P and K than untreated soil. Fumigation with carbon disulphide produced a change in the amounts of Ca, Mg, K, and Mn in extracts of treated soil. In addition this chemical decomposed in the soil to give sulphate. Carbon disulphide has the same effect on nitrifying bacteria in soil as other partially sterilizing agents mentioned before.

Chloropicrin ($CCl_3.NO_2$) used as a nematocide and later as a fungicide, is now considered more fungicidal and herbicidal than any

of the other common soil fumigants. It decomposes in soil to give chlorides (47). It is extremely effective against fungi, nematodes, insects, weed seeds, and has a slight reducing effect on numbers of actinomycetes in the soil (36). Most of the other common fumigants, D-D (dichloropropene propane mixture), methyl bromide, and ethylene dibromide, have by far their greatest use for controlling nematodes (47, 60). D-D eradicates only nematodes, although claims have been made that it will destroy some soil fungi. In a few instances, bacterial soil infestations have been eliminated by D-D (55). Some other relatively new soil fumigants are also recommended for partial sterilization of soil. V-C13 nemacide is used for destroying the free nematodes and also those that subsequently emerge from infected roots. It may or may not destroy nematodes within roots. Terraclor is ineffective against the genera Fusarium, Phythium, Phytophthora, and Thielaviopsis, but is very effective against Rhizoctonia, Selerotinia, Sclerotium, Plasmodiophora, Streptomyces, Tilletia and Botrytis (60). Mylone, a new chemical, is used for controlling soil-borne fungi, nematodes and weeds. Attention has been focused on controlling cyst-forming nematodes such as the golden nematodes (Heterodera rostochiensis) and the more recently discovered and appreciated soybean-cyst nematode (Heterodera glycines). Telone is mentioned as a potent eradicant of cyst-forming nematodes.

Use of heat or chemicals for sterilization unfortunately tends to change the chemical and nutritional properties of soils rather markedly. Sterilization of soil, by either irradiation with an electron beam or by x-ray treatment, has been tried (53, 54, 61).

Soil so sterilized can be assumed to be virtually free from chemical change and should lend itself readily to studies with, for example, pure cultures of plant pathogens, nitrogen fixers, or soil-borne viruses. The action of electrons or x-rays on microbial cells is less drastic than heat or chemical treatments, which generally result in the indiscriminate denaturation of cellular enzymes (61). It has been reported (73) that soil sterilized by irradiation still exhibited phosphatase and urease activity, and was neither toxic nor did it supply extra nutrients to plants. It does, therefore, provide the plant physiologist with a method for the study of uptake of inorganic nutrients from sterile soils which possesses all the purely chemical and structural features of a natural soil.

MATERIALS AND METHODS

1. Soil Samples

The experimental soils were collected from three fields growing alfalfa. General information about these soils is given in Appendix 1. In the fall of 1961, Nonay and Webber fields were sampled from an area approximately an acre in size. Growth was generally poor but patches of good growth were scattered throughout the fields.

The Boje field was sampled in the summer of 1962. It has an undulating topography and generally the growth of alfalfa was poorest in the low areas and best on the high areas. Soil samples were collected from both the good and poor areas. Figure 1 illustrates the marked difference between growth for the two areas.

FIGURE 1. Alfalfa from Boje poor area (centre) and from Boje good area (each side).

Sampling was done to a depth of 0-12 inches, the samples were bulked, passed through a shredder, then through a 0.5-cm. mesh screen, and mixed before being used for greenhouse experiments. For the laboratory investigations, about five pounds of soil were crushed, passed through a 2-mm. sieve, and thoroughly mixed. During these operations, all possible precautions were taken to prevent mixing of one soil with another, thereby keeping contamination to a minimum. Additional soil samples were taken for laboratory study from Nonay and Webber fields from the following depths: 0-12, 12-24 and 24-36 inches.

2. Greenhouse Experiment on Nonay Soil (Leith sandy loam) and Webber Soil (Peace Hills fine sandy loam)

The first greenhouse experiment in this study was conducted on Nonay and Webber soils in the fall of 1961 and spring of 1962. It involved the following treatments.

A - Check.

B - Partial steam treatment.

C - Prolonged steam treatment.

D - Vapam ($CH_3.NH.CS.SNa,2H_2O$) treatment.

F - 60-80-60[1] + minor elements (m.e.).

G - Partial steam treatment + 60-80-60 + m.e.

H - Prolonged steam treatment + 60-80-60 + m.e.

I - Vapam treatment + 60-80-60 + m.e.

K - Insecticide (D.D.T.) + 60-80-60 + m.e.

The treatments were replicated five times. Vapam treatment was carried out by applying one ounce of vapam 4-S (diluted in one or two

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[1] 60 lb.N/ac.; 80 lb. P_2O_5/ac.; 60 lb. K_2O/ac.

gallons of water) to 3.5 cu. ft. of soil and mixed thoroughly. Following this, the soil was immediately placed in plastic bags which were kept closed for two or three days. It was then spread on benches for about one week until dry and free from odour. Ten pounds of soil were placed in glazed clay crocks for all treatments and brought to field capacity with distilled water. The partial and prolonged steam treatments were carried out using steam at 240°F. (15 pounds pressure) for two hours and twelve hours respectively. For each treatment soil samples were placed in sterilized flasks plugged with cotton for laboratory study.

The nitrogen, phosphorus and potassium fertilizers were added in solution one inch below the soil surface, in the form of NH_4NO_3, $CaH_4(PO_4)_2$, and KCl respectively. The micronutrients B, Mn, Zn, Cu, S, Mo, Co, Fe and Cl were added as a nutrient solution approximately every thirty days (Appendix 2). For treatment K, 50 per cent wettable D.D.T. was used as an insecticide at the rate of 1/2 teaspoon per ten pounds of soil and mixed thoroughly.

Grimm alfalfa seeds were placed in petri dishes, moistened, sprinkled with "Nitragin AB" legume inoculant and allowed to germinate. The seedlings were planted on January 5 and January 8, 1962, in the Webber and Nonay soils respectively. One week later the plants were thinned to eight per pot. The pots were brought up to weight (field capacity) once a week with distilled water, and between weighings they were watered when observations indicated the need. The percolation of water through the pots, although almost completely avoided, was collected in plastic saucers placed underneath the pots and then washed

back into the pots. Artificial light was provided from 8 a.m. to 11:30 a.m. and 12 midnight to 4:30 a.m. until May 22, 1962, after which no additional light was used. Three cuts were removed, each at the early bloom stage of the best stand. Plants were harvested each time by cutting at approximately one inch above the soil surface followed by drying overnight in an oven at 65^{o}C and then weighed. The experiment was terminated on May 30, 1962, when the soil was dumped from each pot on to big sheets of paper and all nodules were collected carefully from the soil mass. The soil was then washed carefully from the roots and all nodules were excised and collected in petri dishes containing distilled water. These nodules were used for the laboratory study as described below. The root mass was examined, air dried, and weighed.

3. Sand Culture Experiment using Nodules from Plants Grown in Nonay and Webber Soils

These greenhouse experiments were started on July 30 and August 20, 1962, for Nonay and Webber soils respectively. These were a continuation of the greenhouse experiment discussed in the previous section. Plants were grown in a glass apparatus described by Leonard (42). Briefly, it consisted of two glass chambers connected by a glass tube. The upper chamber contained washed coarse sand and the lower one was filled two-thirds full with nitrogen-free nutrient solution (No. 2 Appendix 3). This solution was drawn up into the sand through a wick. The entire assemblies were sterilized before starting the experiment. The alfalfa seeds used were surface sterilized with alcohol and hydrogen perioxide and were aseptically planted by inserting them into the sand with sterile forceps. Fifteen seeds were planted in each assembly and after germination

thinned to eight. When the young plants had reached a height of approximately one inch, they were inoculated with a suspension of Rhizobium obtained from one replicate of each treatment. These inocula were prepared by selecting and crushing healthy nodules and then growing them on appropriate nutrient agar (No. 1, Appendix 3). Ten ml. of this suspension were added to each assembly by placing several drops of the fluid around the base of each plant. Dust was reduced to minimum in the greenhouse by washing the benches and floor. The nutrient solution in the lower chamber of the apparatus was replaced periodically and care was taken not to splash solution or sand from one assembly to another. The plants were harvested at the early bloom stage, dried at 65°C overnight and weighed. Roots were removed from the sand, washed, examined for nodules, air dried, and weighed.

4. Preliminary Greenhouse Study using Soil from Areas of Good and Poor Alfalfa Growth in the Boje Field

This experiment on the Boje soil was started on June 26, 1962. The reasons for conducting this experiment were to determine whether similar growth differences would occur in the greenhouse as in the field and to further test whether the poor growth was due to a sulphur deficiency. Nine pounds of soil were placed in plastic pots and the following treatments were given, using three replications.

A - Check.

B - 12 lb. sulphur/ac. in the form of Na_2SO_4.

C - 12 lb. sulphur/ac. in the form of $(NH_4)_2SO_4$.

Planting and watering were carried out as previously described. The first crop was harvested on October 7 and the second crop on November 21,

1962. The plants were dried at 65°C for overnight and weighed.

5. Greenhouse Experiment using Soil Leachates from Boje Good and Poor Areas

The second greenhouse experiment on Boje soil also involved soil collected from the good and poor areas. It was carried out using glass apparatus as described in part 3. Fifty g. of soil were shaken with 500 ml. of distilled water for three hours, allowed to settle, and then filtered using suction. This leachate was placed in the lower chamber with and without nutrient solution (No. 3, Appendix 3) as described below. The treatments replicated four times, were as follows.

(a) 60 ml. nutrient solution + 240 ml. distilled water.

(b) " " " " + 240 ml. leachate from soil of good area.

(c) " " " " + 240 ml. of leachate from soil of poor area.

(d) " " distilled water + 240 ml. leachate from soil of good area.

(e) " " " " + 240 ml. leachate from soil of poor area.

Fifteen Grimm alfalfa seeds were planted on September 23, 1962, in sterilized sand in the upper chamber of each apparatus. They were later thinned to eight per jar. Subsequently, the above solutions were added to the lower chamber when necessary. Artificial light was supplied starting September 28, 1962, from 6 a.m. to 10 p.m. Temperature and humidity controls in the greenhouse were set at 70°F and 20 per cent respectively. Plants were harvested on November 21, 1962, dried at 65°C overnight and weighed. The roots were examined for nodules.

6. Greenhouse Experiment using Soil from Areas of Good and Poor Growth in the Boje Field

This was a more elaborate experiment than the preliminary experiment outlined under part 4. The following treatments[1] were replicated six times in two randomized block experiments with one seeded to alfalfa and the other to barley.

A - Check.

B - Partial steam treatment.

C - Prolonged steam treatment.

D - Vapam treatment.

E - 60-80-60 + m.e.

F - Formaldehyde (HCHO) treatment.

N - Nemagon (CH_2Br-$CHBr$-CH_2Cl) treatment.

Formaldehyde (0.8 per cent in water) was applied at the rate of 1/2 gallon per cu. ft. soil. The soil was mixed thoroughly, placed in plastic bags for three or four days and spread on benches to dry. Soil was then placed in plastic pots (8.5 lb. in each pot) and leached with 1000 ml. of distilled water to remove the residual formaldehyde.

Nemagon 170 was applied at the rate of one-half gallon (one part diluted with 50 parts of water) per cu. ft. of soil and the same operation was done as described in the case of vapam treatment.

Steam treatment of the soil was carried out in glazed clay crocks and the soil was then transferred to sterilized plastic pots provided with plastic saucers. After sterilization small samples of soil were immediately placed in previously sterilized flasks plugged with cotton, for laboratory

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[1]The author is indebted to Dr. W.P. Skoropad, Plant Pathologist, University of Alberta, for helping in carrying out these treatments.

study.

In one experiment, 15 sprouted Grimm alfalfa seedlings were planted on October 19, 1962. After two weeks, the plants were thinned to eight in each pot. In the second experiment, 15 to 20 seeds of Gateway barley were placed in each pot on October 31, 1962, and a week after germination the number was reduced to ten. Watering with distilled water was done using the same procedure as described earlier in part 2. Artificial light was provided from 6 a.m. to 10 p.m. Temperature and humidity in the greenhouse were set at 70^{o}F and 20 per cent respectively. The first barley crop was harvested on December 10, 1962, when the plants were beginning to form heads. The second barley crop was planted on January 3, 1963. In order to break the soil crust the surface soil in the pots was stirred, again 60-80-60 was applied before planting the second crop. The crop was harvested on February 8, 1963. The first cut of alfalfa was taken on January 29, 1963 when approximately one third of the plants were at the early bloom stage. Fertilizer at the rate of 0-80-0 was applied after harvesting the first cut of alfalfa and the second crop was harvested on February 27, 1963. The barley and alfalfa plants were dried at 65^{o}C overnight and weighed.

7. Laboratory Studies

Chemical analyses were done on Nonay and Webber soils collected from 0-12, 12-24 and 24-36 inch depths. The pH values of the soil paste (80) were measured with a Beckman model H-2 pH meter. Exchangeable cations, cation exchange capacity, easily soluble phosphorus and percentage of organic matter were determined by methods as outlined by Atkinson *et al*. (5). Presence of lime was detected by adding dilute HCl directly to the soil. Total nitrogen of the finely-ground soil samples was determined by the

Kjeldahl method. The ammonia distilled was collected in 4 per cent boric acid solution and titrated using a mixed indicator composed of methyl red and bromcresol green.

The oven dry plant material was cut into small pieces and then ground in a small Wiley mill to pass a 20 mesh sieve. Nitrogen was determined by the Kjeldahl method and phosphorus by the metavanadate method (32). The nitrate-supplying power of the soil was determined by the method of Eagle and Matthews (19). Briefly, three cm. of powdered vermiculite were placed in a glass tube with a small hole in the bottom and a cap at the other end. A 10-g. sample of air-dry soil was placed on top of the vermiculite and a second layer of vermiculite was added on top of the soil. Sufficient distilled water (approximately 7 ml.) was added to wet the upper layer of vermiculite, the soil, and the upper portion of the lower layer of vermiculite. The soil was then incubated at 35°C in a high relative humidity for one week. After incubation the soil was leached with 100 ml. distilled water in 10 ml. aliquots and the nitrate was measured by the phenoldisulphonic acid method (Appendix 5). The initial nitrate content of the soil, determined by leaching a separate sample of the soil with 100 ml. distilled water, was subtracted from the nitrate content obtained after incubation. The difference was the nitrate that had accumulated during incubation.

Nitrifying ability of the soil was determined by using ammonium medium (No. 4, Appendix 3). Inocula of soil were added to test tubes of this medium and incubated at 35°C for periods varying from one to five weeks. For the soil sterility test media No. 5 and 6 (Appendix 3) were used with three replications for each soil. The numbers of microorganisms in the Nonay and Webber soils were determined by the dilution method using media

No. 5 and 6 (Appendix 3) and two replications. Biological activity of the soil was determined by measuring the quantity of CO_2 liberated by the soil (58).

For the determination of haemoglobin, the nodules were excised from the alfalfa roots, taking one pot at a time, and collected in a petri dish containing distilled water. The total number of nodules, and the number of big branched nodules were counted, washed, air dried for fifteen minutes on blotting paper and weighed. They were then crushed in a mortar with an excess of sodium dithionite and transferred to a centrifuge tube with 5 ml. of cold pyridine in three aliquots. After thorough mixing, the mixture was centrifuged at 1000 x G for ten minutes. The supernatant liquid was poured into a 10-ml. volumetric flask. Another 4-ml. aliquot of cold pyridine was added, mixed, and centrifuged as before. The supernatant liquid was again added to the volumetric flask and the volume made up to the 10-ml. mark with cold pyridine. The absorbance of this solution was immediately determined with a Beckman model B spectrophotometer at a wave length of 550 mμ. The amount of haemoglobin was calculated in terms of haematin from a calibration curve (Figure A-2, Appendix 4).

RESULTS AND DISCUSSION

I GREENHOUSE EXPERIMENT ON NONAY AND WEBBER SOILS

1. Characterization of Soils

Chemical analyses indicate that the Nonay and Webber soils are different in many respects (Table 1). For example, a comparison of the surface soils reveals that the Webber soil is higher in organic matter, total nitrogen, cation exchange capacity, and exchangeable Ca and H, but lower in easily soluble phosphorus and Ca:Mg ratio than the Nonay soil.

2. Growth Relationships

The results of the greenhouse experiments which were concerned with the effects of soil sterilization, fertilizer, and insecticide on the top growth and root production of alfalfa for the Nonay and Webber soils are given in Table 2. The yields are averages of three cuts and the root observations were made following the third cut. All values are averages of five replications.

(a) Yields of tops

For the Nonay soil, insecticide and fertilizer treatments (K,F) had no effect on yield while plants treated with vapam (D,I) yielded significantly higher amounts of dry matter than those in other treatments (Table 2, Figure 2). Prolonged steam treatment (C) also increased the yield of alfalfa significantly over the check. However, prolonged steam treatment plus fertilizer was not significantly different from fertilizer alone (H vs. F).

For the Webber soil also, the insecticide had no effect and fertilizer increased yield only in the case of prolonged steam treatment (Table 2). Partial and prolonged steam treatment (B,C,G,H) resulted in significantly

TABLE 1 SOME CHEMICAL ANALYSES OF NONAY (LEITH SANDY LOAM)

AND WEBBER (PEACE HILLS FINE SANDY LOAM) SOILS

Depth in.	pH	Exchangeable cations me./100 g.					Exchange capacity me./100 g.	Ca Saturation %	Ex. Ca / Ex. Mg	Easily soluble phosphorus ppm.	Total nitrogen %	Organic matter %
		Ca	Mg	Na	K	H						
Nonay soil												
0-12	6.2	5.0	1.0	0.05	0.06	1.3	8.0	63	4.9	54	0.048	0.8
12-24	6.2	5.3	1.5	0.02	0.05	0.9	7.7	69	3.5	29	0.029	0.4
24-36	8.1	5.5[1]	1.3	0.02	0.05	-	6.9	80	4.2	15	0.022	0.4
Webber soil												
0-12	5.8	6.1	0.9	0.03	0.06	2.0	9.1	67	6.7	29	0.102	2.4
12-24	6.0	4.9	0.8	0.03	0.04	1.4	5.7	85	6.0	42	0.041	1.1
24-36	6.2	3.3	0.7	0.04	0.04	1.1	4.5	75	4.6	32	0.009	0.8

[1] Value calculated by difference : free lime present in this horizon.

TABLE 2 DRY MATTER YIELDS OF TOPS AND ROOTS OF ALFALFA GROWN IN NONAY AND WEBBER SOILS IN THE GREENHOUSE

Treatment	Nonay soil						Webber soil					
	Dry matter in tops (sum of three cuts)		Dry matter in roots (After third cut)		Top:root		Dry matter in tops (sum of three cuts)		Dry matter in roots (After third cut)		Top:root	
	g./pot	stat.[1] sig.	g./pot	stat. sig.	ratio	stat. sig.	g./pot	stat. sig.	g./pot	stat. sig.	ratio	stat. sig.
A Check	11.55	a	6.88	a	1.73	b	9.44	a	9.22	a	1.03	bcd
B Partial steam	13.30	ab	13.16	d	1.03	a	12.30	bcd	15.49	cd	0.80	a
C Prolonged steam	15.68	b	10.55	bc	1.71	b	16.89	e	14.24	c	1.19	d
D Vapam	20.35	c	7.99	a	2.64	c	11.37	abc	9.57	ab	1.19	d
F Fert.[2]	13.57	ab	7.98	a	1.72	b	10.54	ab	10.53	ab	1.00	bc
G Partial steam and fert.	14.03	ab	13.47	d	1.04	a	13.89	d	15.81	cd	0.89	ab
H Prolonged steam and fert.	15.21	b	12.07	cd	1.28	ab	19.50	f	16.50	d	1.18	cd
I Vapam and fert.	22.58	c	8.91	ab	2.55	c	13.38	cd	11.18	b	1.20	d
K Insecticide (DDT) and fert.	13.81	ab	9.20	ab	1.55	ab	12.49	bcd	10.71	ab	1.18	cd

[1] In this and subsequent tables "stat. sig." means "statistical significance at the 0.05 level" as determined by Duncan's Multiple Range Test (79). Mean followed by letter "a" is significantly different from those not having "a"; those followed by "b" are significantly different from those not having "b", etc.

[2] Fert. = 60-80-60 lb./ac., plus micronutrients.

FIGURE 2. Responses to vapam treatment prior to the third harvest.

higher amounts of dry matter. The increases from prolonged steam treatments over their respective check treatments, viz. 79 per cent (C vs. A) and 85 per cent (H vs. F), are certainly noteworthy. The vapam treatment had no significant effect on the yield of alfalfa which is in marked contrast to the results obtained for the Nonay soil.

The significantly higher yields of alfalfa caused by the vapam treatment on the Nonay soil and by the steam treatment on the Webber soil are striking and may be attributed to biological and/or chemical changes in the soil. It is known that steam treatment of soil tends to increase the amounts of soluble phosphate, potash, manganese, zinc, calcium, magnesium, copper, boron, sulphur, etc. (1, 21, 40, 46, 56). It also eliminates many soil-borne pathogens (36, 55, 88). Furthermore, it is known that soils rich in organic matter show a higher total salt content after steaming than soils low in organic matter (56). No information is available in the literature concerning the release of nutrients in soil by vapam treatment. It is a temporary soil sterilant and is effective in controlling nematodes, soil fungi, etc. (60). In view of the foregoing, it is apparent that steam treatment of soils can have a marked effect on

the release of nutrients. Perhaps this is the reason for the large increase in yield following steam treatment for the Webber soil in particular. However, this aspect is puzzling because the fertilizer treatment (F) supplied what were considered to be adequate amounts of macro- and micronutrients and yet the responses were not large. The only exception was a significant response from fertilizer when it was applied to the prolonged steam treatment for the Webber soil (H vs. C).

There is the possibility of course that one or more of the nutrients may not have been applied in optimum amount. Nevertheless, substantial responses were not obtained from macro- and micronutrients applied under field conditions (65) for either soil, thereby supporting the greenhouse results. These greenhouse and field experiments suggest that steam treatment affected some important factor other than release of nutrients even though the latter was involved to some degree. The marked and prolonged responses from vapam treatment of the Nonay soil suggest even more that some factor other than nutrient release was involved because it is difficult to visualize that this chemical would be highly active in this regard since heat effects were not involved.

It is known that both steam and vapam markedly alter the microflora in soil and it is reasoned that this may be an important factor affecting the yield in this study. However, little has been reported in the literature about this particular aspect and consequently only a suggestion can be made until further data are available. Since no significant increase in the yield of alfalfa was obtained by vapam treatment of the Webber soil, it can be concluded either that the biological effects on the growth of alfalfa are of little importance

for this particular soil or that the organic matter inactivated the vapam to some degree.

The insecticide treatment was included because a few sweet clover weevil larvae (Sitona cylindricollis) were found in the Webber soil but it is evident that the yield responses from the vapam and steam treatment were not due to destruction of insects.

(b) Root growth

Root growth in Nonay soil was stimulated by both partial and prolonged steam treatments (Table 2). The roots in these treatments (B,C,G,H) were much more branched and prolific than those in other treatments. The response patterns for root production in Webber soil were similar to those observed for Nonay soil (Table 2). However, a significant increase in root growth did not necessarily result in a corresponding increase in top growth. By arranging the top and root yields in ascending order of magnitude, or by calculating the shoot:root ratios, it was evident that no consistent relationship existed between top growth and root growth for the experiment as a whole. For example, in Nonay soil vapam plus fertilizer (I) and vapam alone (D) produced the highest yields but the roots were similar to those for the check which had the lowest root weight of all treatments. For Webber soil, partial steam treatment (B) gave the third highest root weight but only the sixth highest yield. Prolonged steam treatment (C) gave the fourth highest root weight and the second highest yield. Hence it cannot be concluded that plants made better top growth because of more extensive root development or vice versa. This is evidence that there were factors other than root development that affected top growth.

The steam treatments tended to produce a fibrous root system which is somewhat atypical for alfalfa. Examination of roots from all treatments and roots from the field revealed no symptoms of disease due to pathogenic organisms[1].

Top:root ratios were reasonably constant within treatments and significant differences occurred between treatments. For Nonay soil partial steam treatment resulted in the lowest ratio (1.04) and vapam treatment the highest (2.60). In Webber soil the partial steam treatment again showed the lowest ratio (0.84) and vapam the highest (1.19). Addition of fertilizer did not affect top:root ratios in either soil.

3. Nutrient Content of Plants (Table 3)

(a) Nitrogen concentrations

The application of fertilizer (F) on Nonay soil, significantly reduced the nitrogen percentage of the plants. This was also true when fertilizer was applied to the prolonged steam treatment (H vs. C). However, no explanation can be given for this effect. Vapam (D) also significantly decreased the nitrogen content of the plants when no fertilizer was applied and this was probably due to growth dilution. This is suggested because of the high dry matter yield. Partial steam treatment increased the nitrogen percentage of the plants when the Nonay soil was fertilized.

The nitrogen concentration of the plants on Webber soil was significantly increased by both partial and prolonged steam treatment in the non-

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[1]The author is indebted to Dr. W.P. Skoropad, Plant Pathologist, University of Alberta, for making these examinations.

fertilized soil (B,C) and by the prolonged steam treatment where fertilizer was applied (H vs. F). Fertilizer also increased nitrogen percentage of plants (F vs. A, I vs. D). The addition of fertilizer to the steam treated soils (G, H), however, caused no change in the nitrogen percentage of the plants over their respective checks (B,C). The high nitrogen content of plants in the steam treated soils, particularly the prolonged steam treated, was undoubtly due to the release of nitrogen as a result of the heat treatment. Various workers have reported that ammonium is formed during the process of sterilization. Generally there is not a sudden increase in ammonium concentration, but rather a gradual increase with time (13, 14, 40, 68). The amount of ammonium ultimately formed is governed by the organic matter content of the soil, being highest for high organic matter soils (46, 72).

(b) Nitrogen yields (Table 3)

On Nonay soil, significantly higher nitrogen yields resulted from all treatments except fertilizer and insecticide which had no effect. On Webber soil the nitrogen yield increased significantly due to the steam treatments (B,C, vs. A: G,H, vs. F). Fertilizer also increased the nitrogen yield (F vs. A).

(c) Phosphorus concentrations (Table 3)

The pattern of response was similar in the two soils. Vapam had no effect on phosphorus percentage in the plant. Where no fertilizer was applied partial steam treatment decreased the phosphorus level, whereas on the fertilizer treated soils both steam treatments resulted in an increase. In addition, fertilizer increased phosphorus percentages in plants grown on steam treated soils. This suggests that in both soils

TABLE 3 NITROGEN CONCENTRATIONS, NITROGEN YIELDS, AND PHOSPHORUS CONCENTRATIONS FOR FIRST CUT OF ALFALFA GROWN IN NONAY AND WEBBER SOILS IN THE GREENHOUSE

Treatment	Nonay soil						Webber soil					
	Nitrogen in tops				Phosphorus in tops		Nitrogen in tops				Phosphorus in tops	
	%	stat. sig.	g./pot	stat. sig.	%	stat. sig.	%	stat. sig.	g./pot	stat. sig.	%	stat. sig.
A Check	2.65	bcd	0.112	a	0.24	bc	1.83	a	0.054	a	0.23	b
B Partial steam	2.70	cd	0.168	bc	0.19	a	2.60	bcd	0.145	cd	0.20	a
C Prolonged steam	2.85	d	0.194	c	0.25	cd	2.86	d	0.181	e	0.22	b
D Vapam	2.31	a	0.164	bc	0.23	b	1.73	a	0.068	a	0.22	b
F Fert.	2.32	a	0.146	ab	0.24	bc	2.52	bc	0.127	bc	0.25	bc
G Partial steam and fert.	2.61	bcd	0.177	bc	0.26	d	2.52	bc	0.160	de	0.28	cd
H Prolonged steam and fert.	2.53	abc	0.186	bc	0.28	e	2.70	d	0.210	f	0.29	d
I Vapam and fert.	2.26	a	0.184	bc	0.22	b	2.32	b	0.113	b	0.22	b
K Insecticide (DDT) and fert.	2.39	ab	0.146	ab	0.23	bc	2.58	bcd	0.140	bcd	0.24	b

partial steam treatment caused some fixation of phosphorus and thus made it less available to the plants. This is at variance with the results of various workers who have reported increases in soluble phosphorus as a result of steam treatment of soil (40, 46, 48). Lapensee (40) also found that water soluble phosphate was increased by steam treatment of soil for 20 hrs. under 12 lb. pressure. The soil was water saturated before the steam treatment. Malowany and Newton (46), in a study of the changes caused by steaming four Alberta soils, also found an increase in water soluble phosphorus. These results were obtained by autoclaving the soils in one gallon crocks at 15 to 17 lb. pressure for 45 min. on the first day and for 30 min. on the second. This treatment, in comparison to that used by some other workers, was very mild. However, Robinson (67) reported some fixation of phosphorus as a result of heating soil for two hours at 5 lb. pressure. In the present study it appears that mild steam treatment resulted in some fixation of soil phosphorus but that fertilizer phosphorus was rendered more available by the steam treatment.

4. Nodule Characteristics

(a) Numbers and weights of nodules

Fertilizer additions appear to have increased the total number of nodules for all treatments in both soils, but due to the variability within treatments none of the differences were statistically significant (Table 4,5). Stimulation of legume nodule numbers by low levels of added ammonium has been reported (28, 66).

On Nonay soil (Table 4) vapam produced significantly higher numbers of large, branched nodules on both fertilized and unfertilized soils, while partial steam treatment produced lower numbers (not significant

on the fertilized soil, however). On the vapam treated Nonay soil fertilizer significantly decreased the number of large nodules (I vs. D). For the fertilized Webber soil, vapam (I) significantly increased the number of large nodules (Table 5). The same trend was evident on the non-fertilized soil for the vapam treatment but difference was not significant (D vs. A). It is evident that partial and prolonged steam treatments had a depressing effect on the number of large nodules formed in both soils. The accumulation of ammonium due to steam treatment and its conversion to nitrate probably depressed the number of large nodules. It can be concluded that the depressing effects of these available forms of nitrogen are more marked on the number of large nodules than on the total number of nodules. Owing to the presence of more organic matter in Webber than in Nonay soil, more nitrogen was released by steam treatment in the former and consequently the number of large nodules was reduced to a greater extent than on the latter soil. Numerous papers have been published on the depressing effect of nitrogen on production, size, and function of nodules (95). Also it has been reported that a low carbohydrate-nitrogen ratio in the plant, leading to inadequate carbohydrate supply in the roots, may be responsible for decreased nodulation and this condition is more likely to occur in the presence of abundant ammonium and nitrate nitrogen (4). It has already been mentioned that partial steam treatment causes some phosphorus fixation in the soils studied. This possibly had an indirect effect on the number of large nodules. Van Schreven (28) reported that the number and density of nodules are greatly stimulated by phosphorus, and nodule growth is greatly increased. The increase in nitrogen fixation per unit weight of nodules was correlated with the phosphorus content of the nodules. Furthermore,

TABLE 4 NODULE DATA AT TIME OF THIRD CUT FOR ALFALFA GROWN IN NONAY SOIL IN THE GREENHOUSE

Treatment	Total number of nodules		Number of large branched nodules		Weight of nodules		Mean size of nodules		Total haemoglobin		Haemoglobin concentration in fresh nodules	
	no./pot	stat. sig.	no./pot	stat. sig.	g./pot	stat. sig.	mg./nodule x 10^{-3}	stat. sig.	µg./pot	stat. sig.	µg./g	stat. sig.
A Check	230	a	45	bc	0.31	a	1.5	a	- [1]	-	- [1]	-
B Partial steam	273	a	7	a	0.31	a	1.2	a	21	a	62	a
C Prolonged steam	203	a	24	ab	0.38	a	1.8	ab	30	a	80	a
D Vapam	396	a	79	d	1.03	b	2.6	b	144	b	120	b
F Fert.	276	a	30	ab	0.49	a	1.7	ab	34	a	54	a
G Partial steam and fert.	341	a	8	a	0.43	a	1.1	a	30	a	53	a
H Prolonged steam and fert.	382	a	20	a	0.56	ab	1.4	a	28	a	57	a
I Vapam and fert.	527	a	56	c	1.03	b	1.9	ab	126	b	111	b
K Insecticide (DDT) and fert.	308	a	24	ab	0.52	a	1.6	a	38	a	61	a

[1] Samples lost.

TABLE 5 NODULE DATA AT TIME OF THIRD CUT FOR ALFALFA GROWN IN WEBBER SOIL IN THE GREENHOUSE

Treatment	Total number of nodules		Number of large branched nodules		Weight of nodules		Mean size of nodules		Total haemoglobin		Haemoglobin concentration in fresh nodules	
	no./pot	stat. sig.	no./pot	stat. sig.	g./pot	stat. sig.	mg./nodule x 10^{-3}	stat. sig.	µg./pot	stat sig.	µg./g.	stat. sig.
A Check	98	a	53	abc	0.89	bcd	9.6	e	129	c	148	bc
B Partial steam	208	a	22	a	0.39	a	1.9	a	33	a	84	a
C Prolonged steam	199	a	36	ab	0.61	ab	3.1	ab	67	ab	115	ab
D Vapam	148	a	64	bc	1.15	d	8.2	de	187	d	161	c
F Fert.	113	a	64	bc	0.76	bc	6.9	cd	101	bc	134	bc
G Partial steam and fert.	220	a	27	a	0.44	a	3.5	ab	48	a	105	ab
H Prolonged steam and fert.	200	a	24	a	0.46	a	3.0	ab	45	a	107	ab
I Vapam and fert.	287	a	120	d	1.45	e	5.8	c	201	d	140	bc
K Insecticide (DDT) and fert.	214	a	71	c	0.95	cd	4.8	bc	118	c	123	abc

he quoted the work of Poschenrieder and Lesch, who found that with soybeans the number of nodules was increased by potash. Van Schreven also quoted the work of Hilder and Spencer who reported that large, branched, pink nodules were associated with plants receiving sulphur, while untreated plants were small and had scattered unbranched nodules with greenish pigmentation near the base of the nodules.

It will be noticed that the insecticide (D.D.T.) had no effect on the nodules in both Nonay and Webber soils. However, some workers have found that nodulation of inoculated clover was drastically affected by applying 10 lb./ac. of D.D.T. (8). Vincent (28) also found that most of the insecticides caused some inhibition of crown nodulation.

On Nonay soil, the total weights of nodules from the vapam treated soil (I,D) were significantly higher than the check (Table 4). Furthermore, the mean size of the nodules was significantly higher for the vapam treatment on the non-fertilized soil (D vs. A). On the Webber soil, the total weight of nodules was decreased by the steam treatments while vapam produced a greater weight of nodules than all other treatments (Table 5). These differences were significant, however, only on the fertilized soil with the exception of treatment B. When fertilizer was added to the vapam treated soil the weight of the nodules increased significantly, while the mean size of the nodules decreased (I vs. D). A significant decrease in the mean size of the nodules was also observed after partial and prolonged steam treatments (B,C vs. A; G,H vs. F). These results may be attributed to the same factors as discussed previously.

(b) Haemoglobin content

Fertilizer had no effect on haemoglobin content in either soil (Tables 4,5). In Nonay soil the vapam treatments (D,I) produced

approximately 4-5 times more haemoglobin per pot and twice the concentration of haemoglobin in the nodular material than the other treatments (Table 4). For the Webber soil the amount of haemoglobin per pot was significantly reduced by partial and prolonged steam treatment and significantly increased by the vapam treatment as compared to the check (Table 5). However, the haemoglobin concentration in the fresh nodules was reduced significantly as compared with the check only by partial steam treatment on the non-fertilized soil. It is apparent that the haemoglobin concentration in the nodules on both soils varies more or less according to the weight of nodules, i.e. the higher the weight of nodules produced, the greater the concentration of haemoglobin. In summary, Tables 4 and 5 show these important features (i) haemoglobin concentration of nodules for Nonay soil was generally lower than for Webber soil (ii) vapam treatment resulted in a marked increase in haemoglobin concentration for Nonay soil, and (iii) total haemoglobin was decreased by steam treatment and increased by vapam for Webber soil.

(c) Nodule-yield relationships

Figure 3 shows some interesting features concerning the relationship between haemoglobin concentration, weight of nodules, number of large nodules and yield of alfalfa on Nonay and Webber soils. Because fertilizer had no statistically significant effect on these properties, the bar heights of each treatment in Figure 3 are means of treatments with and without fertilizer. It will be noted for Nonay soil that the yield of alfalfa and the haemoglobin concentration in the nodules increased together. A similar relationship was also observed by Jordan

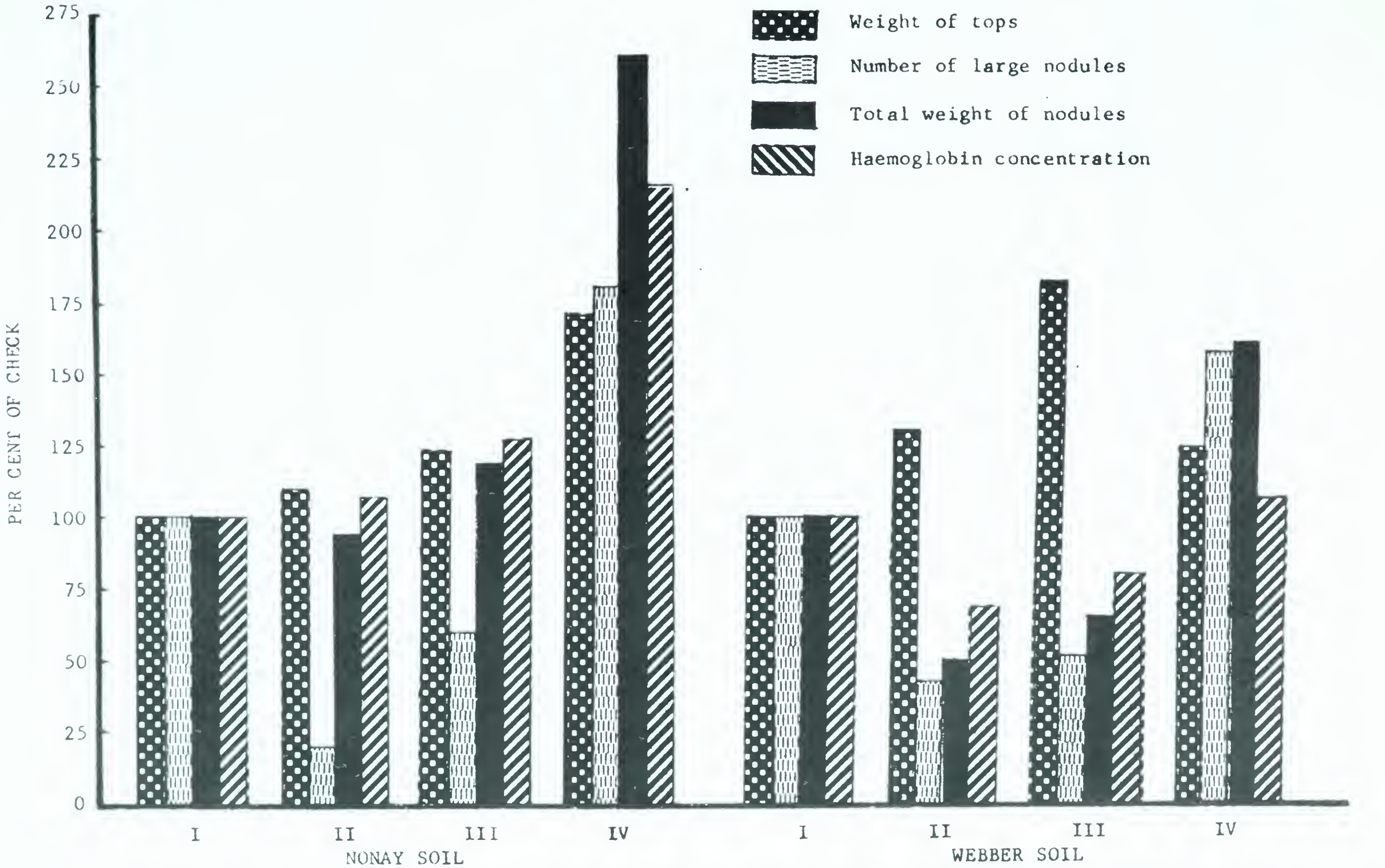

FIGURE 3. The relationship between yield, number of large nodules, total weight of nodules and haemoglobin concentration for alfalfa grown in Nonay and Webber soils in the greenhouse. I - check, II - partial steam treatment, III - prolonged steam treatment, IV - vapam treatment.

and Garrard (35). It is noteworthy that the nodule weight for Nonay soil is positively correlated with plant yield (Table 6) which is in accordance with the finding of Masefield (48). Furthermore, haemoglobin concentration and number of large nodules are also significantly correlated

TABLE 6 RELATIONSHIP OF YIELD WITH NUMBER OF LARGE NODULES, WEIGHT OF NODULES AND HAEMOGLOBIN CONCENTRATION

Comparison	Correlation coefficient	
	Nonay soil	Webber soil
Yield vs. number of large branched nodules	+0.68[1]	-0.36
Yield vs. weight of nodules	+0.83[2]	-0.40
Yield vs. haemoglobin concentration	+0.86[2]	-0.31

[1]Significant, $P < 0.05$ level.

[2]Significant, $P < 0.01$ level.

with plant yield. Thus, it is suggested that on Nonay soil the higher yield obtained was at least partially due to the large production of nodules containing high amounts of haemoglobin, thereby increasing nitrogen fixation. It is not clear, however, why the plants did not respond to the application of 60 lb. of nitrogen per ac. (F, Table 2). Furthermore, the addition of fertilizer to the vapam treatment did not further increase yields significantly (I vs. D, Table 2).

Consequently, the reason for the increased yields resulting from the vapam treatment must be more involved than simply an increase in nitrogen fixation. For the Webber soil the foregoing relationships were not obtained (Figure 3) and it is evident that high nodule weight and high haemoglobin concentration did not increase top growth. It is important to note also that although greater amounts of haemoglobin, weights of nodules, and numbers of large nodules were found for plants grown in the check of Webber soil as compared to the check of Nonay, the yield from the former soil was lower (Tables 2, 4, 5). It appears that these properties are not suitable criteria for explaining increased yields on all soils. It is suggested that nitrogen was not the limiting factor for the growth of alfalfa on the Webber soil.

5. Inoculation Study

Table 7 shows the results of the sand culture experiment using inocula of Rhizobium strains from nodules of the alfalfa grown in the various treated Nonay and Webber soils. The appearance of the plants prior to harvesting are shown in Figure 4. During the time span between taking photographs and harvesting the plants, it was noted that growth differences had disappeared between treatments for Nonay soil probably due to contamination. Furthermore, plants in the Nonay experiment had been grown for ten weeks while those in the Webber experiment were harvested after eight weeks. Owing to these factors, not much reliance can be put on the data shown in Table 7. Consequently, the photographs form a better basis for discussion. Roots and nodules were also examined, and it was noticed that in the Nonay sand culture experiment the plants inoculated with Rhizobium cultures from the vapam

TABLE 7 YIELDS OF ALFALFA GROWN IN SAND CULTURE INOCULATED WITH RHIZOBIUM CULTURES FROM NODULES OF PLANTS PREVIOUSLY GROWN IN NONAY AND WEBBER SOILS GIVEN A VARIETY OF TREATMENTS

Treatment	Nonay		Webber	
	Dry matter in tops g./pot	Dry matter in roots g./pot	Dry matter in tops g./pot	Dry matter in roots g./pot
X Control [1]	1.82	0.74	0.02	0.09
A Check	3.13	3.92	0.10	0.13
B Partial steam	2.67	1.72	0.47	0.20
C Prolonged steam	1.72	1.85	1.69	1.01
D Vapam	3.92	5.95	1.60	0.72
F Fert.	4.59	4.42	0.19	0.32
G Partial steam and fert.	4.32	1.79	1.44	0.89
H Prolonged steam and fert.	3.70	1.38	1.59	0.91
I Vapam and fert.	2.82	1.50	1.91	1.21

[1] Not inoculated

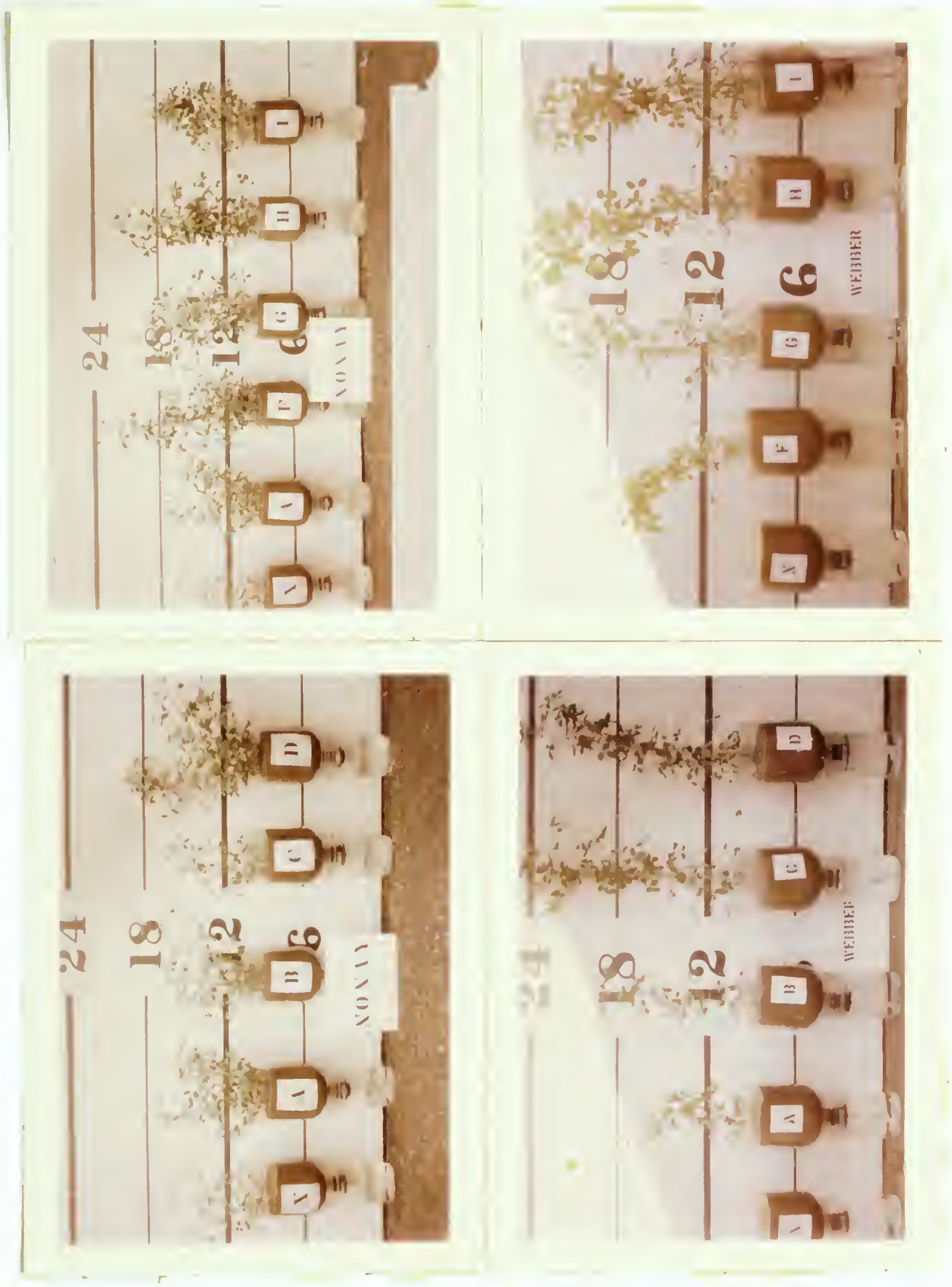

FIGURE 4. Growth of alfalfa in sand-culture experiments using Rhizobium inocula from plants grown in Nonay and Webber soils. Treatments are control (no inoculum); A-check; B-partial steam treatment; C-prolonged steam treatment; D-vapam; F-fertilizer (60-80-60 + minor element); G-partial steam treatment plus fertilizer; H-prolonged steam treatment plus fertilizer; I-vapam plus fertilizer.

treatments (D and I) had healthy pink nodules. The root system for vapam alone (D) was more extensive than for the other treatments. This is interesting because plants grown in the vapam treated soils in the pot experiment also produced large pink nodules (Table 4). The greenhouse pot and sand culture experiments suggest that for the Nonay soil the vapam treatments favoured those Rhizobium strains capable of producing efficient nodules. In the Webber sand culture experiment, healthy pink nodules were observed only for complete steam and vapam treatments (C,H,D,I). The root systems for these treatments were more extensive than for the other treatments.

In summary it is apparent from Figure 4 that the inocula differed widely in their growth producing ability when applied to plants grown in sterilized sand. For Nonay soil inocula from the vapam (D) and fertilizer (F) treatments were more effective than from the check (A). For Webber soil, inocula from the prolonged steam (H) and vapam (D) treatments were the most effective in producing growth. It is not clear why the vapam treatment produced such effective nodulation and yet did not significantly increase yields for Webber soil in the pot experiment. Nevertheless, it is important to note that this treatment did produce effective nodulation in this soil as indicated by high weight of nodules and high haemoglobin concentration (Table 5). This suggests that even though effective strains were present some factor that the vapam did not control limited yield. These data, even though preliminary in nature, suggest that the various treatments had marked effects on the strains of bacteria infecting the roots. In view of its important implication, this aspect should receive further study.

II GREENHOUSE EXPERIMENT ON BOJE SOILS

1. Characterization of Soils

Throughout the text the terms "good" and "poor" soils are used because growth of alfalfa on these two soils in the field during the summer of 1962 was very good and very poor respectively. It should be noted that the poor soil is considerably higher in nutrients than the good soil (Table 8). For example, total nitrogen, nitrate-nitrogen, and available phosphorus contents of the poor soil are approximately twice the corresponding values for the good soil. Available potassium is also higher in the poor soil, being 168 lb./ac. as compared to 120 lb./ac. for the good soil. The foregoing data suggest that low levels of nitrogen, phosphorus and potassium were not responsible for the poor growth. There is the possibility, however, that some micronutrient was deficient or that some chemical or biological agent inhibited growth of alfalfa. It should also be noted (Table 8) that the poor soil is more acidic (pH 5.5) than the good soil (pH 6.3). The lower pH could conceivably affect growth of alfalfa although this is thought to be rather unlikely but nevertheless should be investigated at a future date.

2. Preliminary Experiment with Alfalfa

Soils from the good and poor areas were sampled (0-12 in.) in June, 1962, and the experiment begun on June 26. Sulphur, when applied as Na_2SO_4 to the good soil, increased the yield of alfalfa significantly over the check for the first cut (Table 9). However, the yield was not significantly increased by $(NH_4)_2SO_4$. It appears that responses to sulphur were dependent on the carrier although the reason for this is

not known. However, Cairns and Carson (11) found that sulphur applied as Na_2SO_4 on Grey Wooded soils was more effective in increasing the

TABLE 8 SOME CHEMICAL ANALYSES OF BOJE GOOD (LEITH SANDY LOAM) AND POOR (CODNER SANDY LOAM) SOILS

Soil	pH	Available nutrients lb./ac.			Conductivity	Sulphate in soil extract ppm.	Total nitrogen %
		Nitrate-nitrogen	Phosphorus	Potassium			
Good	6.3	20	45	120	0.5	Nil[1]	0.10
Poor	5.5	40	101	168	0.8	Nil[1]	0.18

[1]Not detectable.

yield of alfalfa on sulphur deficient soils than $CaSO_4$ or elemental sulphur. Application of sulphur to the poor soil as Na_2SO_4 or $(NH_4)_2SO_4$ had no effect on yield. This suggests that sulphur deficiency was not responsible for the poor growth in the field.

It will also be noted from Table 9 that treatment differences and differences between the two soils were not significant for the second cut although there was still a trend for the yields to be lower for the poor soil than for the good soil. This is in contrast to the first cut when all treatments for the poor soil yielded significantly less than for the good soil. This tendency for yields on the two soils to equalize under greenhouse conditions has important implications. It is reasoned that if growth on the poor soil in the field was limited by a deficiency of some element then the differences in growth in the greenhouse between the good

TABLE 9 ALFALFA YIELDS ON BOJE GOOD AND POOR SOILS IN IN THE PRELIMINARY GREENHOUSE EXPERIMENT (MEANS OF THREE REPLICATIONS)

Treatment	Dry weight of tops			
	First cut		Second cut	
	g./pot	stat. sig.	g./pot	stat. sig.
Good soil				
A Check	9.30	b	5.42	a
B Na_2SO_4 [1]	10.33	c	6.52	a
C $(NH_4)_2\ SO_4$ [1]	9.36	bc	4.18	a
Poor soil				
A Check	6.20	a	4.00	a
B Na_2SO_4 [1]	6.02	a	2.87	a
C $(NH_4)_2\ SO_4$ [1]	6.28	a	3.34	a

[1] Added at rates equivalent to 12 lb. sulphur/ac.

and poor soils should have increased with each succeeding cut. However, the reverse occurred suggesting that perhaps some biological effect was involved which was changed by the greenhouse environment. This will be discussed in detail later.

3. Main Greenhouse Experiment

Bulk soil samples (0-12 in.) were collected from the good and the poor areas in the Boje field in September, 1962. Alfalfa was grown in one experiment involving a complete set of treatments on the good and poor soils and barley in a second identical experiment.

(a) Alfalfa yields

Nemagon. The nemagon treatment had no significant effect on the growth of alfalfa on either soil. This treatment was included because it was found that samples collected in the field during the summer of 1962 contained saprophytic, predaceous and parasitic nematodes. In the good soil there were traces of Paratylenchus sp., Tylenchus sp., and Tylenchorhynchus sp.whereas in the poor soil there were traces of Tylenchus sp. and 2000 and 1400 Paratylenchus sp. in duplicate samples[1].

Nematodes of the genus Paratylenchus are also referred to as "pin nematodes" and are known to enter into an ectoparasitic relationship with many plants. They have, however, not been reported as serious parasites of alfalfa. Since nemagon had no significant effect on the yield of alfalfa for either cut on either soil (Table 10), it can be concluded that the presence of nematodes was not responsible for the poor growth in the field.

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[1]The author is indebted to Mr. W.R. Orchard, Plant Pathologist, Experimental Farm, Saanichton, B.C., for this information.

TABLE 10 ALFALFA YIELDS FOR BOJE GOOD AND POOR SOILS IN THE GREENHOUSE (MEANS OF FIVE REPLICATIONS)

Treatment	Dry weight of tops for good soil				Dry weight of tops for poor soil			
	First cut		Second cut		First cut		Second cut	
	g./pot	stat. sig.	g./pot	stat. sig.	g./pot	stat. sig.	g./pot	stat. sig.
A Check	4.78	b	2.68	a	3.49	a	2.31	a
B Partial steam	5.73	c	3.60	c	6.26	c	4.43	b
C Prolonged steam	6.72	d	3.54	c	6.64	c	4.33	b
D Vapam	3.67	a	2.65	a	3.58	a	2.25	a
E Fertilizer[1]	5.55	c	2.94	ab	4.87	b	2.91	a
F Formaldehyde	4.13	ab	3.36	bc	4.62	b	2.82	a
N Nemagon	4.30	ab	2.53	a	3.41	a	2.23	a

[1]60-80-60 lb./ac. plus micronutrients applied before planting and 0-80-0 lb./ac. plus micronutrients following first cut

Vapam. The growth of the first alfalfa cut on the good soil was reduced considerably by the vapam treatment, as compared with the check (Table 10). The plants in this treatment grew slowly following transplanting and behaved as though there was still a residue of vapam in the soil. However, vapam had no significant effect on yield for the second cut. The growth of alfalfa in the poor soil was not affected by the vapam treatment.

Formaldehyde. Increases in yield of alfalfa resulting from formaldehyde treatment were significant only for the second cut on the good soil and the first cut on the poor soil. The significant increases in yield are difficult to interpret.

Fertilizer. Application of 60-80-60 + micronutrients increased the yield of the first cut significantly on both soils. This shows that one of the nutrients supplied in the fertilizer stimulated growth. However, additional 0-80-0 applied immediately after harvesting the first cut did not significantly increase the yield of the second cut on either soil. From this it appears that phosphorus was not the limiting factor. The fact that this fertilizer treatment did not increase yields to nearly the same extent as the steam treatments raises the possibility that some factor in addition to mineral nutrition is involved.

Steam treatment. Steam treatment of both soils resulted in marked yield increases for both the first and second cuts (Table 10). For example, prolonged steam treatment increased yields for the first and second cuts of alfalfa on soil from the good area by 41 and 32 per cent respectively. The comparable figures for the poor soil were 90 and 88

per cent respectively. The partial steam sterilization treatment had a similar effect with yield increases of 20 and 34 per cent for the first and second cuts respectively on the good soil and 80 and 92 per cent on the poor soil. It is known (46, 56, 91) that steam sterilization can have a marked effect on the release of certain nutrients and this factor could have been at least partially responsible for the increased yields in this experiment. A confusing aspect, however, is that the yield increases were much greater for the steam treatments than for the fertilizer treatment. It is possible of course that the latter treatment did not supply some of the nutrients in optimum amounts but the rates applied are regarded as being substantial and yet not within the toxic range. It is important to note at this point that applications of macro- and micronutrients to a similar soil under field conditions failed to give substantial yield increases and that growth remained poor even on heavily fertilized plots. These field applications, although not made to Boje good and poor soils, were applied to an area in an adjoining field and to Nonay and Webber soils discussed earlier. Poor growth of alfalfa is a problem on all these soils. Furthermore, it is surprising that the effects of the steam treatments were so prolonged especially in the case of the partial steam treatment which provided a rather mild heat treatment. It is obvious that additional experiments are required to further elucidate whether the markedly increased yields resulting from steam treatment were due to a chemical or biological effect or both. However, the results certainly suggest that a biological factor is involved.

(b) Barley yields

It will be noted for the first crop and for the average of the first and second crops that the yields were better on the poor soil than on the good soil (Table 11). This is the reverse of what happened with alfalfa (Table 10) indicating that the two crops reacted quite differently in the two soils.

Nemagon. Nemagon had no significant effect on barley yields, although there was a trend towards increased yields (Table 11).

Chemical sterilants. Vapam significantly increased the yields of barley for the first crop on the good soil and for the second crop on the poor soil. Although this significant effect was not observed for the second crop on the good soil and for the first crop on the poor soil, there was still a trend towards increased yields. It is interesting that the formaldehyde treatment increased yield significantly for the first crop of barley on both soils; for the second crop, however, the increase was not significant indicating that the effect was short lived. The increased yield from vapam and formaldehyde may have been due to the elimination of some harmful microorganisms in both soils. This applies especially to fungi, which under some conditions, can produce a toxic effect on plant growth. For example, fungi and other microbes are found growing in close association with plant roots and some produce antibiotic substances (47, 89). If an organism producing such a substance were to become established in close contact with the plant root, it is reasonable to hypothesize that root function might be impaired. Moreover, certain fungi, after continuous growth in the root vicinity, may become increasingly virulent, encroach more and more on the root hairs and outer root cells, and thus adversely

TABLE 11 BARLEY YIELDS FOR BOJE GOOD AND POOR SOILS IN THE GREENHOUSE

(MEANS OF FIVE REPLICATIONS)

Treatment	Dry weight of tops for good soil				Dry weight of tops for poor soil			
	First crop		Second crop		First crop		Second crop	
	g./pot	stat. sig.	g./pot	stat. sig.	g./pot	stat. sig.	g./pot	stat. sig.
A Check	0.91	a	2.02	a	1.83	a	1.70	ab
B Partial steam	3.05	b	2.76	b	3.70	c	2.78	d
C Prolonged steam	2.88	b	3.26	c	3.65	c	3.53	e
D Vapam	2.50	b	2.37	ab	2.26	a	2.06	c
E Fertilizer[1]	1.41	a	2.78	b	1.76	a	2.70	d
F Formaldehyde	2.68	b	2.18	a	2.98	b	1.93	bc
N Nemagon	1.39	a	2.13	a	2.39	ab	1.55	a

[1]60-80-60 lb./ac. plus micronutrients applied before planting first and second crops

affect the plant (47). It is confusing that the responses from vapam and formaldehyde were, considering averages of the two barley crops, greater for the good soil than for the poor soil. This again may be attributed to the fact that the factor which caused poor alfalfa yields in the field had little effect on barley.

Fertilizer. For the first crop of barley on both soils, fertilizer did not significantly increase yields. It appears that either the nutrients supplied in the fertilizer were not limiting or there was some interaction between the fertilizer nutrients and some other factor, thereby preventing yield increases. However, when an additional application of 60-80-60 was applied prior to seeding the second crop, the increases were significant on both soils. This shows that one of the nutrients supplied in the fertilizer stimulated growth.

Steam treatment. Partial and prolonged steam treatment increased yields very substantially for both the first and second crops on both soils. Steam treatment usually eliminates or reduces the numbers of harmful microorganisms and releases soluble nutrients from soils (1, 6, 20, 36, 40, 56, 73, 74, 91). In the present study the increased yields may have been due to either one or both of these effects.

4. Leachate Study

Growth differences between some treatments in the leachate study prior to harvest are shown in Figure 5.

FIGURE 5. Alfalfa growth in sand culture experiment using leachates from Boje good and poor soils. Treatments are: (a) nutrient solution; (b) nutrient solution plus leachate from good soil; (c) nutrient solution plus leachate from poor soil; (d) leachate from good soil and; (e) leachate from poor soil

TABLE 12 YIELDS OF ALFALFA GROWN IN SAND CULTURE INVOLVING LEACHATES FROM BOJE GOOD AND POOR SOILS

Treatment	Dry weights of tops g./pot		
	Mean	stat. sig.	
		5% level	1% level
a Nutrient solution	2.46	c	c
b Nutrient solution + leachate from good soil	2.00	b	bc
c Nutrient solution + leachate from poor soil	1.76	b	b
d Leachate from good soil	0.14	a	a
e Leachate from poor soil	0.10	a	a

Plants grown in leachates without added nutrient solution (d,e) yielded significantly less than the other treatments which received nutrient solution (Table 12). There was also a tendency, although not significant, for the plants to grow more poorly in the leachate from the poor soil than from the good soil (d vs. e). This trend was even more marked in the presence of nutrient solution (b vs. c). Furthermore, plants grown in nutrient solution plus leachate from the poor soil (c) yielded significantly less (one per cent level) than those grown in nutrient solution alone (a) whereas the depressive effect of the leachate from the good soil was not significant at the one per cent level. These results suggest that the leachate from the poor soil contained something which was toxic to the alfalfa plants. Another possibility is that the weak growth in the poor area of the field was due to the action of harmful microorganisms. These may have been leached out of the soil, developed subsequently in the sand culture, and lowered yield. An examination of the root nodules showed that plants grown in nutrient solution alone had healthy roots with no nodules. A few nodules were present in the case of the two leachates plus nutrient solution but the roots were less extensive than those from nutrient solution alone.

In the field it was observed that plants growing in the good area had many healthy pink nodules. However, in the poor area, plants had either no nodules or when present were in the form of a few large clumps of whitish nodules. These and nodules from plants growing in the good area were collected and alfalfa plants growing in sterilized sand were inoculated with suspension of Rhizobium strains obtained from these

nodules. The alfalfa plants showed no differences in growth and in nodule development suggesting that the Rhizobium strains from the two areas were similar. It is evident that in the poor area of the field, some factor affected the activity of the nodule bacteria causing them to produce abnormal nodules. When the plants were grown in sterilized sand culture in the greenhouse, however, the nodule formation was normal.

III MICROBIOLOGICAL STUDIES ON NONAY, WEBBER AND BOJE SOILS

1. Changes in Microflora

(a) Checks

For all four soils, an examination of plates revealed that fungi were dominant although bacteria were also present. There were no visual differences in microflora between Boje good and poor soils. There was no evidence of pathogenic fungi.[1]

(b) Steam treatments

Plating of soil samples taken immediately after treatment revealed that partial steaming greatly reduced the numbers of microorganisms and prolonged steaming nearly eliminated them (Table 13). However, when the steamed soils were stored at room temperature in sterilized cotton-plugged flasks for three weeks and again plated, the numbers (by observation) were greater than those reported in Table 13.

It is apparent from the above results that steaming did not kill all microorganisms. That is, the steam treatment did not completely sterilize the soil but it did change the microflora markedly.

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[1]The author is indebted to Dr. W.P. Skoropad, Plant Pathologist, University of Alberta, for making this observation.

TABLE 13 PLATE COUNTS OF MICROORGANISMS IMMEDIATELY AFTER STEAM AND VAPAM TREATMENTS (MEANS OF SIX REPLICATES)

Treatment	Microorganisms in Nonay soil	Microorganisms in Webber soil
	no./g. ± S.D.	no./g. ± S.D.
A Check	240,000 ± 140,000	660,000 ± 130,000
B Partial steam	8,000 ± 10,000	100 ± 200
C Prolonged steam	110 ± 210	10 ± 13
D Vapam	270,000 ± 290,000	340,000 ± 180,000

(c) Chemical treatments

Nemagon. This treatment applied to the Boje soils did not appear to change the microflora.

Vapam. In all soils, vapam eliminated fungi. Total microbial count was not affected for Nonay soil but was somewhat reduced for Webber soil (Table 13). The surviving bacteria appeared to be largely spore formers and a few actinomycetes were also present.

Formaldehyde. This treatment, used on the Boje soils, appeared to alter the composition of the microflora, but did not eliminate bacteria or fungi specifically.

2. Respiration Studies

Carbon dioxide evolution has been used by certain investigators as

a method of measuring the biological activity of soil. Following the preliminary sterility test, the amount of CO_2 produced in the soils under study was determined after incubation periods of 24, 48 and 72 (or 96) hours. For all soils, the CO_2 formation continued with time, but the amount differed between soils and was significantly affected by the various treatments (Tables 14, 15). The data suggest that it would have been desirable to have continued the experiments for a considerably longer period than 96 hours.

The CO_2 evolved from the partial and prolonged steam treated Webber soil was less than from the check and vapam treated soil. This difference was greatest after 72 hours incubation (Table 14). Because the organic matter content of the Webber soil was higher than the Nonay, possibly a larger amount of substrate was released by steaming and hence rapid development of a new microfloral population. In Nonay soil, on the other hand, the trend was for lower respiration in the partial and prolonged steam treated soils than in the check and vapam treated.

Results obtained for different treatments in the case of Boje good and poor soils (Table 15) proved impossible to interpret. In the good soil the trend of respiration was similar in vapam, formaldehyde and check treatments. However, CO_2 evolved at the end of 96 hours in the prolonged steam treatment was low and in partial steam and nemagon treatments was higher than the check. In the poor soil, the CO_2 evolution from the prolonged steamed soil was significantly higher than all other treatments, followed by nemagon. The respiration in partial steam, vapam, formaldehyde and check treatments was similar.

In summary, the gross appearance of plates, as discussed in the pre-

TABLE 14 EFFECT OF STEAM AND VAPAM TREATMENTS ON RESPIRATION IN NONAY AND WEBBER SOILS

Treatments	mg. CO_2 evolved / 5.00 g. oven-dry soil						mg. CO_2 evolved / 5.00 g. oven-dry soil					
	Nonay soil						Webber soil					
	24 hrs.		48 hrs.		72 hrs.		24 hrs.		48 hrs.		72 hrs.	
	Mean	stat. sig.	Mean	stat. sig.	Mean	stat. sig.	Mean	stat. sig.	Mean	stat. sig.	Mean	stat. sig.
A Check	1.58	c	2.30	c	3.58	d	2.24	a	2.96	a	3.93	a
B Partial steam	0.58	a	0.95	a	1.97	b	2.84	a	3.97	c	5.38	c
C Prolonged steam	1.27	b	1.80	b	1.91	a	2.93	a	3.66	b	4.99	b
D Vapam	2.10	d	2.61	d	3.09	c	2.94	a	3.62	b	3.73	a

TABLE 15 EFFECT OF STEAM, VAPAM, FORMALDEHYDE AND NEMAGON TREATMENTS ON RESPIRATION IN BOJE SOILS

Treatment	mg. CO_2 evolved / 5.00 g. oven-dry soil						mg. CO_2 evolved / 5.00 g. oven-dry soil					
	Good soil						Poor soil					
	24 hrs.		48 hrs.		96 hrs.		24 hrs.		48 hrs.		96 hrs.	
	Mean	stat. sig.	Mean	stat. sig.	Mean	stat. sig.	Mean	stat. sig.	Mean	stat. sig.	Mean	stat. sig.
A Check	1.63	b	2.55	a	5.47	c	1.71	b	3.05	c	5.85	b
B Partial steam	1.45	a	4.19	c	8.19	e	1.02	a	2.03	a	6.47	c
C Prolonged steam	2.13	d	2.59	a	3.59	a	3.66	e	7.63	f	13.87	e
D Vapam	1.94	c	2.41	a	5.19	b	1.91	bc	2.59	b	4.96	a
F Formaldehyde	2.13	d	3.19	b	5.24	b	2.08	c	3.52	d	6.53	c
N Nemagon	2.42	e	4.05	c	6.89	d	2.47	d	4.03	e	7.55	d

vious section was the same for any particular treatment of both good and poor soils. However, the patterns of respiration were different for the two soils. Consequently, it is obvious that no conclusion can be drawn regarding the usefulness of either property for helping to understand the problem under study.

(3) Nitrogen Transformations in Soil

In all soils ammonium was present immediately after treatment and was particularly abundant in the steam treated soils. This is in accordance with the results of other investigators (33, 91). Nitrite was not detected in any soil. Steam, vapam, and formaldehyde treatments prevented nitrification during subsequent incubation for one week (Tables 16, 17). Thus, it appears that these treatments destroyed or temporarily inhibited the nitrifying bacteria in the soil. This is supported by the nitrifying tests conducted on these soils (Table 18). However, nemagon which was used only for Boje soils, did not prevent nitrification. The nitrate was decreased in treated soils, except those nemagon treated. This decrease was substantial only in partial steam treated Nonay soil and may be attributed to nitrate assimilation. This is supported by the fact that the total count of microorganisms was larger for this treatment in Nonay soil than in Webber soil (Table 13). The treated and untreated Boje soil samples collected from pots growing alfalfa and barley in the greenhouse gave positive nitrifying tests (Table 18). It appears that when the soil was used and handled in the process of potting and planting some external contamination occurred, thus hastening the onset of nitrification. Furthermore, nitrifying microorganisms were

TABLE 16 EFFECT OF STEAM AND VAPAM TREATMENTS ON NITRIFICATION IN NONAY AND WEBBER SOILS DURING INCUBATION FOR ONE WEEK AT 35°C

Treatment	Nonay soil			Webber soil		
	Initial NO_3-N ppm.	Final NO_3-N ppm.	Difference NO_3-N ppm.	Initial NO_3-N ppm.	Final NO_3-N ppm.	Difference NO_3-N ppm.
A Check	24	44	+20	15	34	+19
B Partial steam	22	1	-21	2	2	0
C Prolonged steam	6	5	- 1	4	4	0
D Vapam	4	7	+ 3	3	1	- 2

TABLE 17 EFFECT OF STEAM, VAPAM, FORMALDEHYDE, AND NEMAGON ON NITRIFICATION IN BOJE SOILS DURING INCUBATION FOR ONE WEEK AT 35°C

Treatment	Good soil			Poor soil		
	Initial NO_3-N ppm.	Final NO_3-N ppm.	Difference NO_3-N ppm.	Initial NO_3-N ppm.	Final NO_3-N ppm.	Difference NO_3-N ppm.
A Check	18	42	+24	20	48	+28
B Partial steam	20	19	- 1	16	12	- 4
C Prolonged steam	5	2	- 3	13	7	- 6
D Vapam	7	3	- 4	5	2	- 3
F Formaldehyde	3	1	- 2	4	1	- 3
N Nemagon	7	21	+14	8	36	+28

TABLE 18 EFFECT OF STEAM, FORMALDEHYDE, VAPAM AND NEMAGON TREATMENTS ON NITRIFYING ABILITY OF BOJE SOILS

Treatment	Soil[1] immediately after treatment		Soil[2] samples from greenhouse pots		Soil[1] 3 1/2 months after treatment			
	Good soil	Poor soil	Good soil	Poor soil	Good soil		Poor soil	
	Three weeks	Three weeks	One week	One week	One week	Five weeks	One week	Five week
A Check	+	+	+	+	+	+	+	+
B Partial steam	-	-	+	+	± [3]	+	±	±
C Prolonged steam	-	-	+	+	±	+	±	+
D Vapam	-	-	+	+	±	+	±	±
F Formaldehyde	-	-	+	+	±	+	±	±
N Nemagon	+	+	+	+	+	+	+	+

1 Soil stored in sterile flasks after the various treatments.
2 Samples were taken from pots after first barley crop and during first alfalfa crop. Values are averages from 12 pots.
3 Traces of nitrate.

present in the treated Boje soils incubated under aseptic conditions at room temperature for 3 1/2 months, although only a very weak nitrifying capacity was evident even after 5 weeks in the case of the poor soil treated with partial steaming, vapam, and formaldehyde. Perhaps only nitrifying heterotrophs were present in these instances.

SUMMARY AND CONCLUSIONS

The purpose of this project was to study the reason for the poor growth of alfalfa on certain soils in the Stony Plain area. Soils on three farms were sampled, viz., Nonay (Leith sandy loam), Webber (Peace Hills fine sandy loam), Boje good (Leith sandy loam) and Boje poor (Codner sandy loam). Greenhouse and laboratory studies were conducted on these samples.

During the period 1960 to 1962 a greenhouse experiment (23) and field experiments (60) were conducted. These provided certain leads and formed a basis for the present study, which is summarized below.

I ALFALFA GROWN IN NONAY AND WEBBER SOILS IN THE GREENHOUSE

1. Webber soil was considerably higher in total nitrogen and organic matter than was Nonay soil.

2. The application of macro- and micronutrients produced only a modest response on both soils. Vapam treatment on Nonay soil and steam treatments on Webber soil produced marked and prolonged yield increases.

3. Steam treatments produced more branched and prolific root systems than the other treatments. The top:root ratios were reasonably constant within treatments but significant differences occurred between treatments.

4. The nitrogen concentration of plants on Webber soil was greatly increased by steam treatments. Partial steaming of soils resulted in some fixation of soil phosphorus. Fertilizer phosphorus, however, was rendered more available by steam treatments.

5. Both partial and prolonged steaming depressed the number of large nodules for both soils. This effect was more marked on Webber than Nonay soil. Vapam treatment produced greater numbers of large nodules, total

weight of nodules, and haemoglobin per pot than the other treatments for both soils, being particularly marked for Nonay soil.

6. For Nonay soil nodule weight, number of large nodules, and haemoglobin concentration were positively correlated with plant yield. However, no such relationship existed for Webber soil.

7. In general Rhizobium strains from plants grown in treated soil were more effective than from the check as indicated by increased yield of alfalfa inoculated and grown in sand culture. Further more, strains from the plants grown with additions of fertilizer were more effective than those from plants grown without fertilizer.

8. From the above facts it is suggested that the poor growth of alfalfa in the field on the Nonay soil was primarily due to biological effects and on the Webber soil to both biological and nutritional effects.

II GREENHOUSE EXPERIMENT ON BOJE GOOD AND POOR SOILS

1. Poor soil was considerably higher in nutrients than good soil.

2. Nematodes were present in greater numbers in the poor than in the good soil but were probably not responsible for the poor growth in the field judging from the lack of response to nemagon treatment in the greenhouse.

3. Steam treatment greatly increased the yield of alfalfa and barley on both soils. The effect was evident over two cuts of alfalfa and two crops of barley.

4. Application of macro- and micronutrients gave increases in yields of alfalfa on both soils. However, these increases particularly for the alfalfa were not nearly as great as those from the steam treatments. This raises the possibility that some factor in addition to mineral nutrition is involved and that it could be associated with the poor growth of

alfalfa in the field. This important aspect requires further study. Applications of phosphorus and sulphur to the poor soil gave little or no response suggesting that these two elements were not lacking.

5. Barley yields in the greenhouse were better on the poor soil than on the good soil, whereas the reverse was true for alfalfa. The fact that the difference in growth of alfalfa between the good and poor soil was greater for the first cut then for the second suggests that the undesirable condition was not stable under greenhouse conditions. Thus it is evident that the condition which produced unsatisfactory growth of alfalfa in the field on the poor soil was also active in the greenhouse but to a lesser degree.

6. Vapam and formaldehyde treatments tended to increase the yield of barley on both soils but had no effect on the yield of alfalfa.

7. Leachate from the poor soil showed a greater depressive effect on the growth of alfalfa in sand culture than leachate from the good soil. This suggests that the poor soil contained some toxic chemical or organism. It could be the factor that was responsible for the poor growth of alfalfa in the field. This requires further investigation.

III MICROBIOLOGICAL STUDIES

1. Steam treatment of the soils did not kill all microorganisms but did change the microflora markedly. Vapam treatment eliminated fungi while formaldehyde was not effective in this regard but appeared to alter only the composition of microflora. Thus, it is evident that the soils were not completely sterilized by any treatment and this is further substantiated by the respiration study.

2. Nitrification was temporarily inhibited in the soils by partial and

prolonged steam, vapam, and formaldehyde treatments. This suggests that the different treatments did not destroy the mechanism of nitrification in the soil.

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APPENDIX 1

FARM CO-OPERATOR, LEGAL DESCRIPTION, SOIL SUB-GROUP AND SOIL TYPE FOR EACH OF THE SOILS USED IN THIS STUDY

Co-operator	Legal description	Soil sub-group	Soil type
M. Nonay	NW 34-50-27-4	Dark Grey Wooded	Leith S.L.
W.P. Webber	SE 13-53-28-4	Orthic Black	Peace Hills F.S.L.
S. Boje (good soil)	SE 1-51-27-4	Dark Grey Wooded	Leith S.L.
S. Boje (poor soil)	SE 1-51-27-4	Orthic Meadow	Codner S.L.

APPENDIX 2

MINOR ELEMENT SOLUTION (31)

H_3BO_3	2.86 g.
$MnCl_2.4H_2O$	1.81 g.
$ZnSO_4.7H_2O$	0.22 g.
$CuSO_4.5H_2O$	0.08 g.
$H_2MoO_4.H_2O$ (85% MoO_3)	0.02 g.
$CoCl_2$[1]	0.02 g.
Distilled water	1000 ml.

[1]Not included in original formulation.

One ml. of the above solution was applied to each pot approximately once every month.

APPENDIX 3

MEDIA USED IN MICROBIOLOGICAL STUDIES

1. Rhizobium medium (Wageningen medium)

Difco yeast extract	1.0 g.
Mannitol	10.0 g.
K_2HPO_4	0.5 g.
$MgSO_4.7H_2O$	0.25 g.
NaCl	0.1 g.
$CaCO_3$	3.0 g.
Difco agar	20.0 g.
Tap water	1000 ml.

2. Nitrogen-free nutrient solution (Newton, 57)

$Ca(H_2PO_4)_2.H_2O$	3.2 g.
$CaCl_2.2H_2O$	4.7 g.
$MgSO_4.7H_2O$	5.5 g.
K_2SO_4	4.0 g.
$FeCl_3.6H_2O$	trace
Distilled water	18 l.

3. Nutrient solution (Hewitt, 30)

KNO_3	1.0 g.
KH_2PO_4	0.5 g.
NaCl	0.1 g.
$CaSO_4.2H_2O$	0.5 g.
$MgSO_4.7H_2O$	0.5 g.
Ferric citrate	0.02 g.
H_3BO_3	0.5 mg.

Mn (as $MnSO_4$)	0.5 mg.
Zn (as $ZnSO_4$)	0.2 mg.
Mo (as Na_2Mo_4)	0.1 mg.
Cu (as $CuSO_4$)	0.003 mgm. upwards
Co (as $CoCl_2$)	0.006 mgm.
Distilled water	1000 ml.

4. Ammonium medium (Newton, 57)

$(NH_4)_2SO_4$	2.0 g.
K_2HPO_4	1.0 g.
$MgSO_4$	0.5 g.
NaCl	2.0 g.
$FeSO_4$	0.4 g.
$MgCO_3$	5.0 g.
Distilled water	1000 ml.

5. Peptone agar medium (Difco, 18)

Peptone	5.0 g.
Glucose	10.0 g.
Agar	20.0 g.
Tapwater	1000 ml.

6. Nutrient agar medium (Difco, 18)

Nutrient agar	10.0 g.
Glucose	10.0 g.
Tapwater	1000 ml.

APPENDIX 4

DETERMINATION OF PYRIDINE HAEMOCHROMOGEN

About 500 mg. of nodules were excised from alfalfa roots, washed, and air dried. The nodules were then crushed in a mortar with an excess of sodium dithionite ($Na_2S_2O_4$), extracted with cold pyridine, and centrifuged. This was repeated twice. The supernatant liquid was collected and the volume made up to 10 ml. with cold pyridine. Approximately 3 mg. of haemin ($C_{34}H_{32}O_4N_4FeCl$) were weighed in a 50-ml. beaker, mixed with excess sodium dithionite and 0.5 ml. distilled water, and then dissolved in 10 ml. of cold pyridine. One ml. of this solution was diluted to 50 ml. with cold pyridine. A portion (100 mg.) of human haemoglobin was ground with 0.20 g. sodium dithionite, transferred to a 50-ml. volumetric flask with distilled water, and made up to volume. This gave a solution of 2000 ppm. which was then diluted as required with cold pyridine. Absorbance values for these three solutions were determined over the wavelength range from 530 to 565 mμ, using a Beckman model B spectrophotometer.

Wavelength mμ	Absorbance		
	Nodule extract	Human haemoglobin	Haemin
530	0.214	0.093	0.110
535	0.192	0.086	0.099
540	0.204	0.102	0.109
542	0.207	0.125	0.119
544	0.223	0.154	0.131
546	0.238	0.182	0.152
548	0.260	0.211	0.183
550	0.273	0.223	0.202
552	0.271	0.217	0.226
554	0.253	0.177	0.229
556	0.223	0.141	0.205
558	0.184	0.093	0.186
560	0.153	0.077	0.172
565	-	0.034	0.145

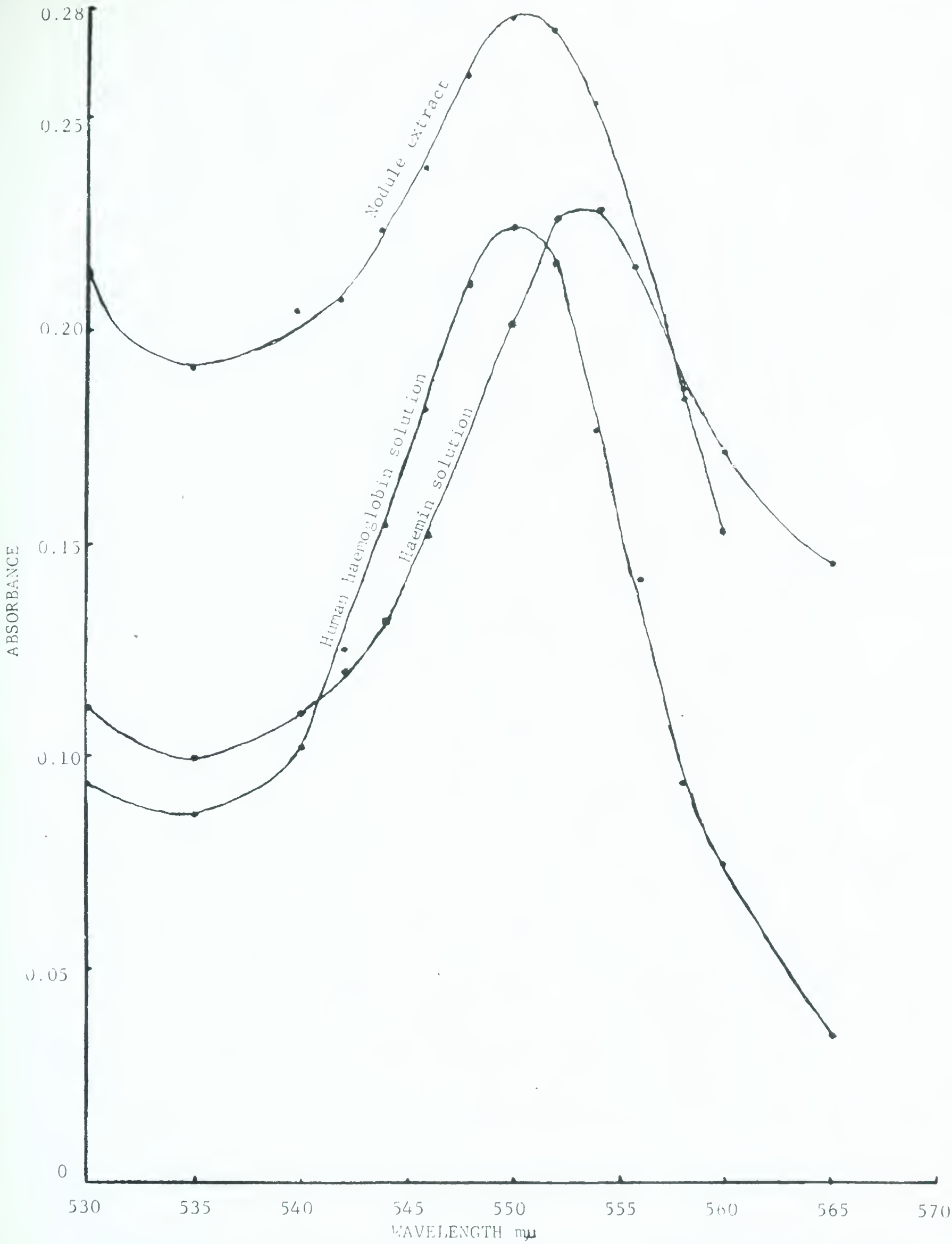

FIGURE A-1. Pyridine haemochromogen absorbance spectra of alfalfa nodule extract, human haemoglobin and haemin.

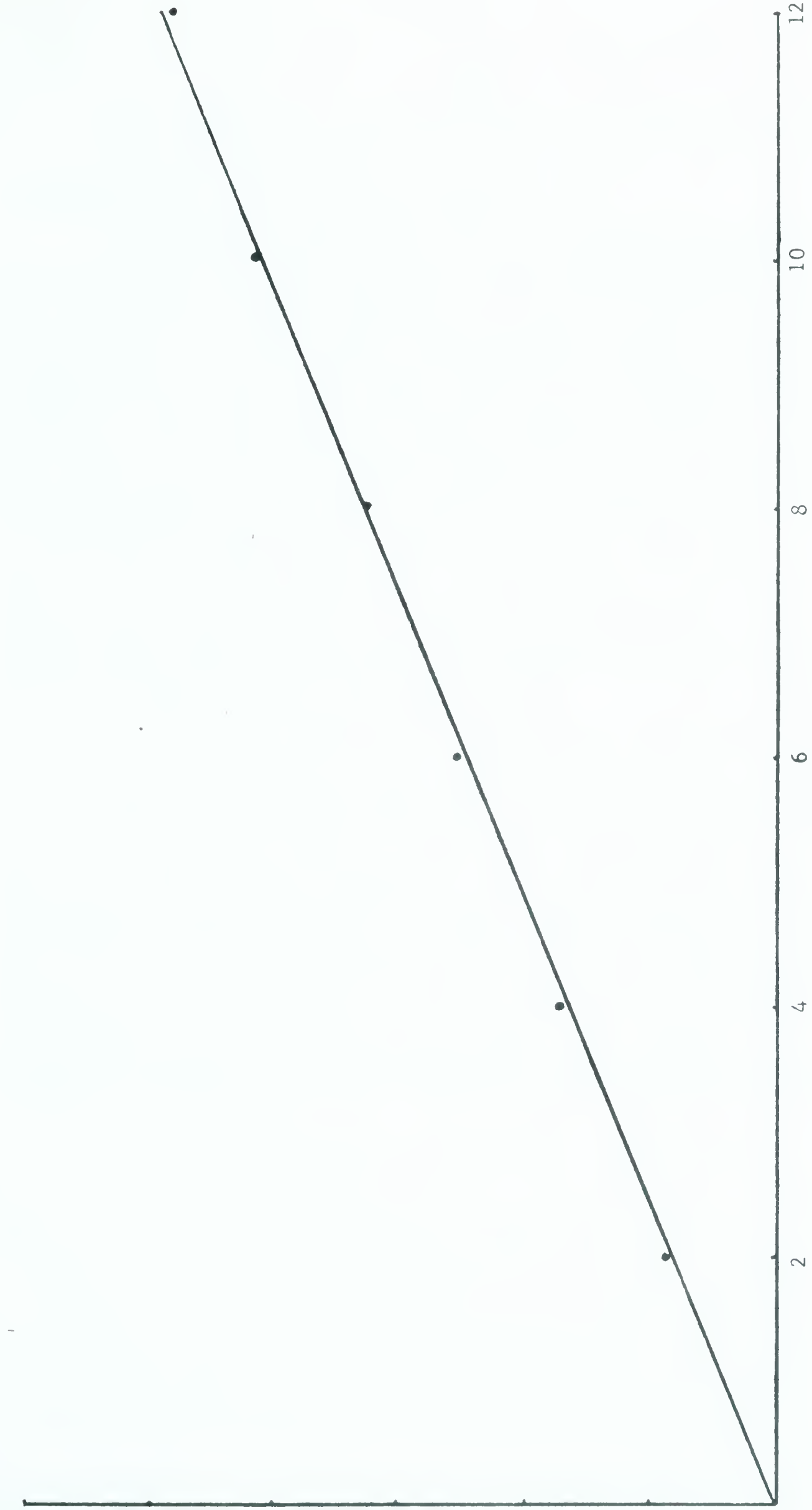

FIGURE A-2. Relationship between haemin concentration and absorbance measured at wavelength 550 mµ.

The wavelength vs. absorbance curves are shown in Fig. A-1. The absorption maxima were between 550 and 554 mμ.

An attempt was made beforehand to determine the effect of temperature and time on the stability of haemin and nodule extract mixed with pyridine. It was found that the pyridine haemochromogen solution kept at freezing temperature was more stable than that kept at room temperature but the absorbance reading decreased gradually with time at both temperatures. However, change in absorbance was almost neglible up to 60 minutes. It was desirable to analyze nodules from one pot at a time, since 30-45 minutes were required for one complete operation. In addition, better results were obtained when nodule haemoglobin was extracted with pure pyridine rather than pyridine plus water.

In preparation of a standard curve 50 mg. of haemin were mixed with 0.1 g. of sodium dithionite and 5 ml. of water and dissolved in cold pyridine. The solution was quantitatively transferred to a 500-ml. volumetric flask and made up to volume with cold pyridine. From this solution, which contained 100 μg./ml. of haemin as pyridine haemochromogen, standards of the following concentrations were prepared: 0,2,4,6,8,10 and 12 μg./ml. Absorbance was determined at wavelength 550 mμ (Fig. A-2).

APPENDIX 5

PREPARATION OF STANDARD CURVE FOR NITRATE DETERMINATION

A standard curve was prepared by taking different aliquots of standard KNO_3 solution (0.7214 g./l.) and making up to volume in 100-ml. volumetric flasks. Nitrate was measured by the phenoldisulphonic acid method by Jackson (32). The standard curve thus prepared is depicted in Fig. A-3.

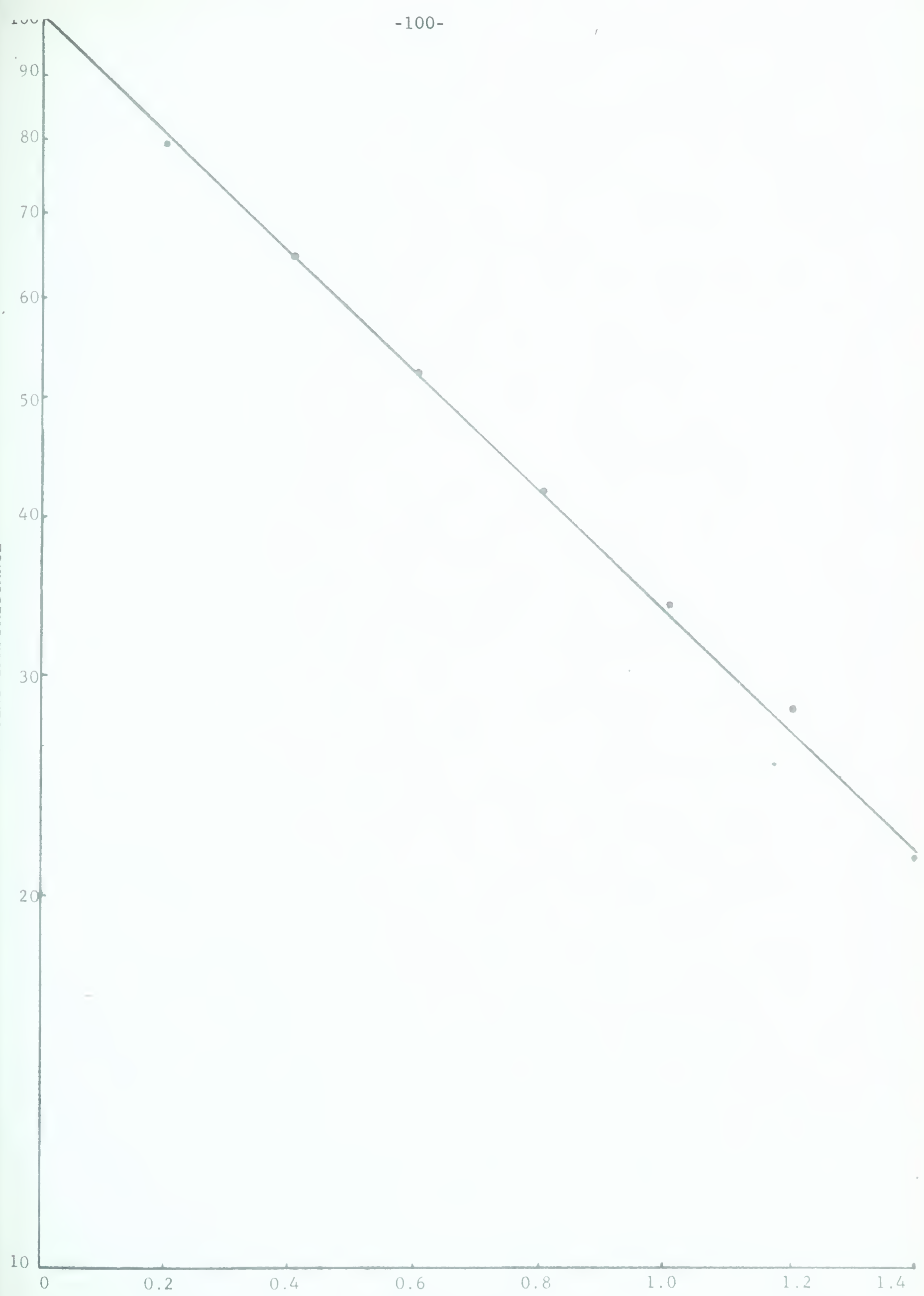

FIGURE A-3. Relationship between nitrate-nitrogen determined by the phenoldisulphonic acid method (32) and transmittance using Bausch and Lomb colorimeter